EFFECT OF TERMINALIA ARJUNA AND EMBLICA OFFICINALIS EXTRACT ON CARDIOVASCULAR SYSTEM IN ALBINO WISTER RATS

Thesis submitted to the

BLDE University, Vijayapur, Karnataka, INDIA

For the award of

DOCTOR OF PHILOSOPHY (MEDICAL)

In

ANATOMY

By

BHEEMSHETTY S. PATIL

Reg No. 10PhD001

Department of Anatomy

Under the Guidance of

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DECLARATION BY THE CANDIDATE

I hereby declare that this thesis entitled "Effect of *Terminalia Arjuna and Emblica Officinalis* extract on Cardiovascular system in albino Wister rats." is a bonafide and genuine research work carried out by me under the guidance of Dr. S. D. Desai, Principal Shridevi Institute of Medical Science & Research Hospital, Tumkur. Karnataka, India. No part of this thesis has been formed the bases for the award of any degree or fellowship previously.

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Certificate

This is to certify that this thesis entitled "Effect of *Terminalia Arjuna and Emblica Officinalis* extract on Cardiovascular system in albino Wister rats." is a bonafide research work carried out by Dr. Bheemshetty S. Patil under our supervision and guidance in the Department of Anatomy Shri. B. M. Patil Medical College, Hospital and Research Center, Vijayapura, Karnataka, India. In the fulfilment of the requirements for the degree of Doctor of Philosophy in Anatomy.

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●●●●●

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ABBREVIATIONS

%	-	Percent
µm	-	Micrometer
µl	-	Micro liter
mmol/L	-	Milimole/Liter
µmol/L	-	Micromole/Liter
nm	-	Nanometre
ml	-	Millilitre
gms	-	Grams
gm	-	Gram
g/dl	-	Grams/Desi litre
g/L	-	Grams/Litre
mg/kg	-	Milligram/Kilogram
IP	-	Indian Pharmaceuticals
B.wt	-	Body weight
HR	-	Hour
IAEC	-	Institutional Animal Ethical Committee
ICMR	-	Indian Council of Medical Research
CPCSEA	-	Committee for the Purpose of Control and Supervision of Experiments on Animals (India)
TLC	-	Thin Liquid Chromatography
Hcl	-	Hydrochloride
Conc	-	Concentration
Na^+	-	Sodium
K^+	-	Potassium
Ca^{++}	-	Calcium
Hb%	-	Haemoglobin Percentage
RBC	-	Red Blood Corpuscles
WBC	-	White Blood Corpuscles
PCV	-	Packed Cell Volume

MCHC	-	Mean Corpuscular Hemoglobin Concentration
TC	-	Total Cholesterol
TGs	-	Trygleseroids
TG	-	Trygleseroid
LDL	-	Low Density Lipoprotein
HDL	-	High Density Lipoprotein
HDL2	-	High Density Lipoprotein 2
HDL3	-	High Density Lipoprotein 3
ILDL	-	Intermediate Low Density Lipoprotein
IDL	-	Intermediate Density Lipoprotein
IHD	-	Ischaemic Heart Disease
FFA	-	Free Fatty Acids
VLDL	-	Very Low Density Lipoprotein
ApoB	-	Apolipoprotein B
Ox LDL	-	Oxidised low density lipoproteins
OxLDLS	-	Oxidatively Modified Low Density Lipoprotein.
MCP1	-	Monocyte Chemotactic Protein-1
CSF	-	Cerebro Spinal Fluid
NO	-	NITRIC OXIDE
TNF Alpha	-	Tumor Necrosis Factor Alpha
MIP1 Alpha	-	Macrophage Inflammatory Protein 1-alpha
VCAM1	-	Vascular cell adhesion molecule-1
ICAM1	-	Intercellular Adhesion Molecule 1
SMCs	-	Smooth Muscle Cells
ROS	-	Reactive Oxygen Spaces
HMG CoA	-	Hydroxy Methyl Glutaryl-CoA Reductase
H_2O	-	Water
H_2O_2	-	Hydrogen Peroxide
O_2	-	Oxygen
ATP	-	Adenosine Tri Phosphate
ADP	-	Adinosine Di Phosphate

Rpm	-	Rotation Per Minute
SGOT	-	Serum Glutamic Oxaloacetic Transaminase,
SGPT	-	Serum Glutamic Pyruvic Transaminase,
ALP	-	Alkaline Phosphatase Level
ALT	-	Alanine Transaminase
LDH	-	Lactate Dehydrogenase
NAD	-	Nicotinamide Adenine Dinucleotide.

ABSTRACT

ABSTRACT

Background*:* Hyperlipidemia is the key factor in the development of atherosclerosis, liver disorders and oxidative stress. For treatment of atherosclerosis and various cardiovascular diseases, much attention has been focused on the use of natural products that have very few side effects. *Terminalia Arjuna (Arjuna) and Embilica Officinalis (Amla)* occupy the pride of place in the context of such medicinal values. *Terminalia Arjuna (Arjuna)* is herbal tree of combretaceae family. Bark of *Terminalia Arjuna (Arjuna)* contains hypolipidemic agents and flavonoids such as arjunolic acid, arjun glycosides, arjunone with rich antioxidative properties. It serves as a cardiac tonic.

Embilica Officinalis (Amla) is also known as *Amla* or Indian gooseberry acts as antihyperlipidemic and antioxidant. Its active ingredients contain tannins, gallic acids and flavonoids.

Aims and Objective: The aim of the present study was to assess the effect of ethanolic extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* on cardiovascular system and histological alteration of heart, elastic artery, muscular artery and coronary artery of albino rats fed with high fat diet.

Materials and methods: Extraction of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* by soxhlet apparatus using 99% ethanol at 60º temp for 22hrs and phytochemical analysis was done. Group 1 served as normal control/Iso-caloric diet. Group 2 fed with hyperlipidemic diet. Group 3 fed with hyperlipidemic diet and treated with *Terminalia Arjuna (Arjuna)* for 21 days. Group 4 fed with hyperlipidemic diet and treated with *Embilica Officinalis (Amla)* 21 days and Group 5 fed with hyperlipidemic diet and treated with *Terminalia Arjuna (Arjuna) and Embilica Officinalis (Amla)* for 21 days

Dose of ethonolic extract of *Terminalia Arjuna (Arjuna)*: (500mg/kg body weight daily) and *Emblica Officinalis (Amla)* (100mg/kg body weight daily).

The results: Significant percent body weight gain in group 2(Hyperlipidemic diet fed rats), however these changes were not observed in hyperlipidemic rats treated with *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla).* There was significant improvement in coronary arterial wall thickness and lumen diameter. Coronary artery showed early fatty changes in hyperlipidemic rats group 2, which brought back to normal in group 3, 4 and 5. (Group 3 rats fed with hyperlipidemic diet and treated with *Terminalia Arjuna (Arjuna),* group 4 rats fed with hyperlipidemic diet and treated with *Emblica Officinalis (Amla)* and group 5 rats fed with hyperlipidemic diet and treated with *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)).*

Conclusion: Results indicate both these two drugs are cardioprotective against hyperlipidemia induced alteration of cardiovascular pathophysiology.

CHAPTER 1

INTRODUCTION

INTRODUCTION:

Hyperlipidemia shares

remarkably in the manifestation and development of atherosclerosis and coronary heart diseases (CHD)[1]. Atherosclerosis causing to coronary artery have presumed a serious disease. It is the prime cause of mortality and morbidity worldwide[2]. Atherosclerosis is a multifactorial disease and about 250 different risk factors have been recognized[3]. Among the several factors high fat diet, unhealthy life style, smoking, family history of hypertension and increased LDL levels are the culprits for the onset of CHDs[1]. Epidemiological studies have revealed a positive significant correlation between Coronary Artery Disease (CAD) and plasma cholesterol concentration[4].Intake of diet with high saturated fat and cholesterol has been proved to be the predominant factor in the progress of atherosclerosis. Experimental studies have proved high fat diet may be connected with increased oxidative stress in mammals[5].

Reducing levels of increased lipids mainly low density lipoproteins (LDL) and triglycerides by dietary interventions or by the drug could reduce the risk of CHD[1].

Synthetic anti hyperlipidemic drugs like statin and synthetic antioxidant like probicol are drug of choice to cure atherosclerosis and its associated complication[6]. Even though statins have been proved effective in lowering increased LDL levels have found side effects[6]. Statins are fundamentally enzyme inhibitor that is HMG CoA.

It is likely that modern medicinal system is curing on one hand and causing side effects on the other hand[7]. Nowadays enhanced interest in natural products has encouraged the search for new hypolipidemic drugs and therapies from these natural sources. Many herbal medicinal products are much efficient in lowering increased lipid levels in body with very low side effects[1].

Hence, for treatment of atherosclerosis and various cardiovascular diseases, much attention has been focused on the use of natural products that have very few side effects[8]. *Terminalia Arjuna (Arjuna) and Embilica Officinalis (Amla)* occupy the pride of place in the context of such medicinal values. Recently there has been renewed interest in these plants because of its multimode cardioprotective activities.

Terminalia Arjuna (Arjuna) (Family: Combretaceae), used in traditional medicine for treating ulcers, wound healing[9], and also for antibacterial[10], antimutagenic/ anticarcinogenic[11], antioxidant and hypocholesterolemic activities[12].The active constituents of *Terminalia include tannins, triterpenoids* saponins (arjunolic acid, arjunic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, oligomericproanthocyanidinspolyphenols, calcium, magnesium, zinc and copper[13].

*Terminalia Arjuna(Arjuna)*acts on cardiac muscles and improves pumping action of heart. It is used as a cardiac tonic[14]. As *Emblica Officinalis (Amla)*, a strong antioxidant and found to have influences on the regulation of lipid metabolism[15].

In search of relative safer alternative compound to fight against cardiovascular risks and diseases especially in case of exposure to high dietary fat consumption, the present study has undertaken to find out cardioprotective efficacy of *Terminalia Arjuna(Arjuna)*and *Emblica Officinalis(Amla)*on experimental model

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CHAPTER 2

AIM AND OBJECTIVES OF STUDY

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Aim:

To assess the effect of ethanolic extract of *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* on the cardiovascular system of albino Wistar rats fed with hyperlipidemic diet.

Objective:

To study the effect of ethanolic extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* in hyperlipidemic rats on

a. Lipid profile
b. Liver function
c. Histology/architecture of Atrium, Ventricle, and vessels
d. Vascular Integrity

HYPOTHESIS:

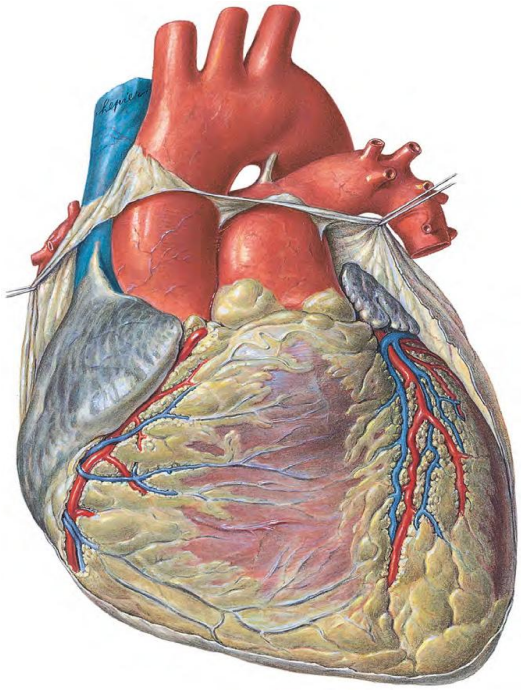
Hyperlipidemic diet

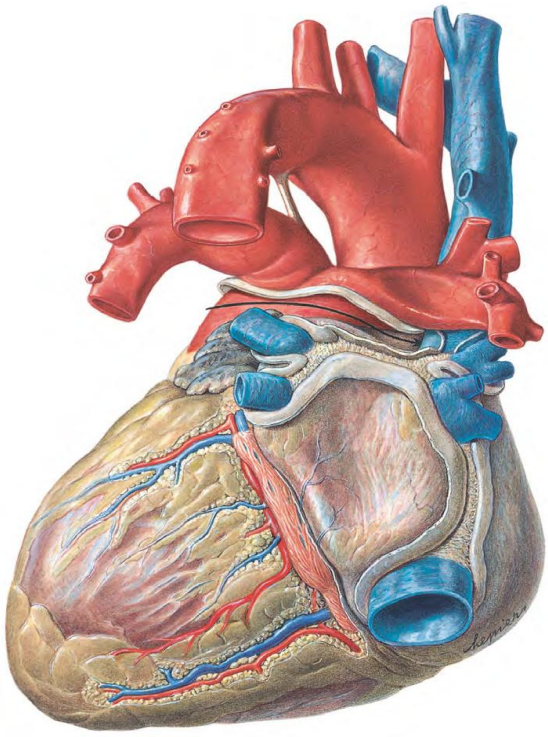
1. Hyperlipidemic diet influences metabolic functions
2. Hyperlipidemic diet makes an adverse effect on the cardiovascular system

Both *Terminalia Arjuna* (Arjuna) and *Emblica Officinalis (Amla)* protect cardiovascular integrity against hyperlipidemia

CHAPTER 3

REVIEW OF LITERATURE





HEART ANTERIOR VIEW **HEART POSTERIOR VIEW**

Anatomy of Cardiac musculature:

Cardiac muscle is present only in the heart, and in the walls of large vessels where they enter the heart. It consists of a branching network of individual cells that are linked electrically and mechanically to function as a unit. Compared with skeletal muscle, cardiac muscle is much less powerful but far more resistant to fatigue. It is provided with a continuous supply of energy by several blood vessels (Coronary artery) around the fibres, and plenty mitochondria within them. Cardiac muscle differs structurally and functionally from skeletal muscle in some important aspects. That is, for example, intrinsically capable of rhythmic contraction, with a rate and strength which is nevertheless responsive to hormonal and autonomic nervous control[1].

Development of Heart:

The cardiac myocytes differentiate from the splanchnic coelomic cells of the pericardium initially subjacent to the endoderm. Myogenic activity starts at the early stage of 10, 22 days gestation, when the embryo has 4 somites. At the same time presumptive cardiac myocytes express contractile proteins like myosin, actin, troponin and other[2].

The cardiac myocytes do not conect with their adjacent to form a syncytium as occurs in skeletal muscle, but remain mononucleated, branched cells connected via intercellular junctions[2].

Anatomy of Myocardium:

In a normal adult the myocardium, the muscular component of the heart, constitutes the bulk of its tissues. It consists predominantly of cardiac muscle cells, which are usually 120 μm long and 20–30 μm in diameter[3].

Every cell has one to two large nuclei, lodge the central part of the cell, but in skeletal muscle has multiple, peripherally situated nuclei. These cells are branched at their ends, and the branches of neighbour cells are so tightly associated that the compound microscopic appearance is on a network of branching and anastomosing fibres. All cells are bound together by elaborate junctional complexes, the intercalated[3].

Fine fibrocollagenous connective tissue is found between cardiac muscle fibres. Although this is equivalent to the endomysium of skeletal muscle, it is less regularly organized because of the complex three-dimensional geometry imposed by the branching cardiac cells. Multiple capillaries and some nerve fibres are found within this layer. Scracthy connective tissue, equivalent to the perimysium of skeletal muscle, separates the larger bundles of muscle fibres,

and is particularly well developed near the condensations of dense fibrous connective tissue that form the 'skeleton' of the heart[3].

The ventricles of the heart are collected of spiralling layers of fibres which run in various directions. Consequently, histological sections of ventricular muscle naturally contain the profiles of cells cut in a variety of orientations. A progressing arrangement of cardiac muscle fibres is obtained only in the papillary muscles and trabeculae carneae3

Gross features of vessel walls:

Artrial and veneous blood vessels, irrespective of size, without capillaries and venules, have walls consisting of three concentric layers (tunicae). The tunica intima, is the innermost layer. The endothelium is the main component, lines the entire lumen vascular, including the endocardium, and the lymph vessels. The tunica media is made of muscle (Smooth muscle) tissue, elastic fibres and collagen. This is the thickest layer in arteries, the media is absent in capillaries and is comparatively thin in veins. The tunica adventitia is the outer layer of the vessel, and consists of connective tissue, nerves and vessel capillaries vasa vasorum. It links the vessels into the surrounding tissues. Vessels change in the relative thicknesses and detailed compositions of their layers and, in the smallest vessels, the number of layers represented[4].

Elastic/ Large arteries:

The best example for elastic artery is aorta and its largest branches are brachiocephalic, common carotid, subclavian and common iliac arteries are also called large elastic arteries which conduct blood to the medium-sized distributing arteries. The tunica intima is made of an endothelium, resting on a basal lamina, and a subendothelial connective tissue layer. The innermost layer or endothelial cells are flat with prominent nuclei, elongated and polygonal in outline, with their long axes parallel to the direction of blood flow. The subendothelial layer of

blood vessel is well developed, consists of elastic fibres and type I collagen fibrils, fibroblasts and small, smooth muscle-like myointimal cells. The hindmost adhered lipid with age and in an ultimate form, this feature contributes to atherosclerotic (fatty) changes in the tunica intima. Wall thickening of the tunica intima progresses with age and is more marked in the distal than in the proximal segment of the aorta[4].

Internal elastic lamina is prominent, sometimes divides and lies between tunica intima and tunica media. The elastic lamina is smooth, measures about 1μm in thickness, and, with the elastic lamellae of the tunica media, is stretched under the effect of systolic pressure, recoiling elastically in diastole. The elastic arteries thus have effect of sustaining blood flow despite the pulsatile cardiac output. They also smooth out the cyclical pressure wave. Tunica media has a markedly layered architecture, in which fenestrated layers of elastin alternate with interlamellar smooth muscle cells, collagen and fine elastic fibres. The position is very regular, such that each elastic lamella and adjacent interlamellar zone is regarded as a 'lamellar unit' of the media. In the human aorta there are approximately 52 lamellar units, measuring about 11μm in thickness. Number and thickness of lamellar units increases during postnatal development, from 40 at birth. The adventitia is well developed. In addition to collagen and elastic fibres, it contains flattened fibroblasts with extremely long, thin processes, macrophages and mast cells, nerve bundles and lymphatic vessels[4].

Smooth muscular arteries:

Smooth muscular arteries are identify by the dominance of smooth muscle in the tunica media. The tunica intima consists of an endothelium, similar to that of elastic/large arteries, which overlies on a basal lamina and subendothelial connective tissue. The internal elastic lamina muscular artery is a distinct, thin layer, sometimes duplicated and sometimes absent. It is

arranged into wavy folds as a result of contraction of smooth muscle in the tunica media. Around 75% of the mass of the tunica media consists of smooth muscle cells, which run spirally or circumferentially around the blood vessel wall. The maximum part of the extracellular matrix is therefore less than in large arteries, however, fine, elastic fibres which run mainly parallel to the muscle cells are present. An external elastic lamina, consists of sheets of elastic fibres, forms a decreased compact layer than the internal elastic lamina, and separates the tunica media from the tunica adventitia in the larger muscular arteries. The adventitia is made of fibroelastic connective tissue, and can be as thick as the media in the smaller arteries. The inner part of the adventitia contains more elastic than collagen fibres[5].

CARDIOVASCULAR DISEASES:

Cardiovascular diseases (CVDs) have been reckoned amongst the top reasons for early deaths in the country. By the year 2020, CVDs is expected that CVDs will become the leading cause of death and disability worldwide. One of the major risk factors for developing CVDs is hyperlipidemia, an elevated level of lipid in the plasma. At basal levels, lipids have been reported to perform important functions in the body, but may cause various adverse health effects if present in excess levels[6].

HYPERLIPIDEMIA:

Hyperlipidemia or hyperlipoproteinemia is the condition of abnormally increased levels of any or all lipids and/or lipoproteins in the blood. These lipids include triglycerides, cholesterols, cholesterol esters and phospholipids. They are transported in the blood as a part of large molecules lipoproteins[7].

Hyperlipidemia is a major cause of atherosclerosis and its associated disorders like CHD, peripheral vascular disease and ischaemic cerebro vascular diseases. The causal relationship has

been well established between increased levels of plasma lipids and development of atherosclerotic plaques[8].

Major Lipids in the Body:

Lipids are heterogeneous group of compounds which are relatively insoluble in water and soluble in non-polar solvents such as ether and chloroform[9]. Three major lipids in the body are triglycerides, total cholesterols and phospolipids[9].

1. Triglycerides: This group is better known as neutral fats. As their name suggests, the triglycerides are composed of one molecule of glycerol and connected through ester bonds with three molecules of fatty acids. Triglycerols are water insoluble non polar neutral fats. TGs serve as the body's major fuel storage reserve. TG synthesis mostly occurs in Liver and adipose tissue. The triglycerol produced in liver is packaged with cholesterol, phospholipids and apo lipoprotein, apo-100 to form VLDL and released into blood stream and delivered to the peripheral tissue[10].

2. Cholesterol: It is the major sterol in the body. Liver and intestine are major sites of cholesterol synthesis. It is found in the cell membrane where it helps maintaining cell integrity and fluidity. It also serves as the precursor molecule for the synthesis of other steroids including bile salts (aids in the digestion of fats) and steroid hormones (such as testosterone, estrogen, progesterone, and cortisol). An abnormality in cholesterol levels in the body can lead to atherosclerosis that can lead to myocardial infarction or stroke[7].

3. Phospholipids: Phospholipids are the major class of membrane lipids. They are composed of a glycerol molecule with two fatty acids (a Diglyceride) they can form lipid bilayers because of their amphiphilic characteristic. It performs important function in maintaining cell permeability[11].

Lipoproteins:

Fat absorbed from the diet and fat synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage. The lipoprotein system evolves in transporting fats in the aqueous environment of the plasma[7].

A lipoprotein is a water miscible complex containing center core of hydrophobic, non polar lipid (TGs and cholesterol ester) covered in a hydrophilic coat of polar lipids that is phospholipids, free cholesterol and apolipoprotein[11].

Five main types of lipoproteins are

a) Chylomicrons

b) Very low density lipoprotein (VLDL)

c) Intermediate density lipoprotein (ILDL)

d) Low density lipoprotein (LDL)

e) High density lipoprotein (HDL).

f) Lipoprotein

a) Chylomicrons: These are principal form in which dietary TGs are carried to the tissues. Dietary lipid is absorbed in the small intestine and incorporated into chylomicrons. Triglycerols are gradually removed from chylomicrons by the action of lipoprotein lipase. After losing TGs, chylomicrons become smaller and richer in cholesterol and cholesterol esters. This is called chlyomicron ruminants.

b) VLDL: These are triglycerol rich particles. VLDL are secreted by liver and transported TGs to the peripheral tissues.

c) IDL: These are formed by removal of TGs from VLDL during formation of LDL from VLDL.

d) LDL: These are cholesterol rich particles, formed from IDL by the removal of more triglycerols and apolipoproteins. They become smaller and denser. Oxidized LDL is more atherogenic. Excess cholestrol is present in the form of LDL hence it is called "Bad Cholestrol"

e) HDL: These are of two types HDL2 and HDL3. They transport cholesterol from peripheral cells to the liver, prior to excretion. They increase in size as they circulate via the bloodstream and attract more phospholipids and cholesterol molecules from cells and other lipoproteins. Raised plasma levels of HDL is associated with reduced risk of IHD and seems to have protective effect. Hence it is known as "Good Cholestrol".

f) Lipoproteins: It is synthesized in the liver and has about the same lipid composition as LDL. It has been shown to compete with plasminogen for tissue plasminogen receptors. It also causes proliferation of smooth muscle cells causing generation of clots and atherosclerosis[11].

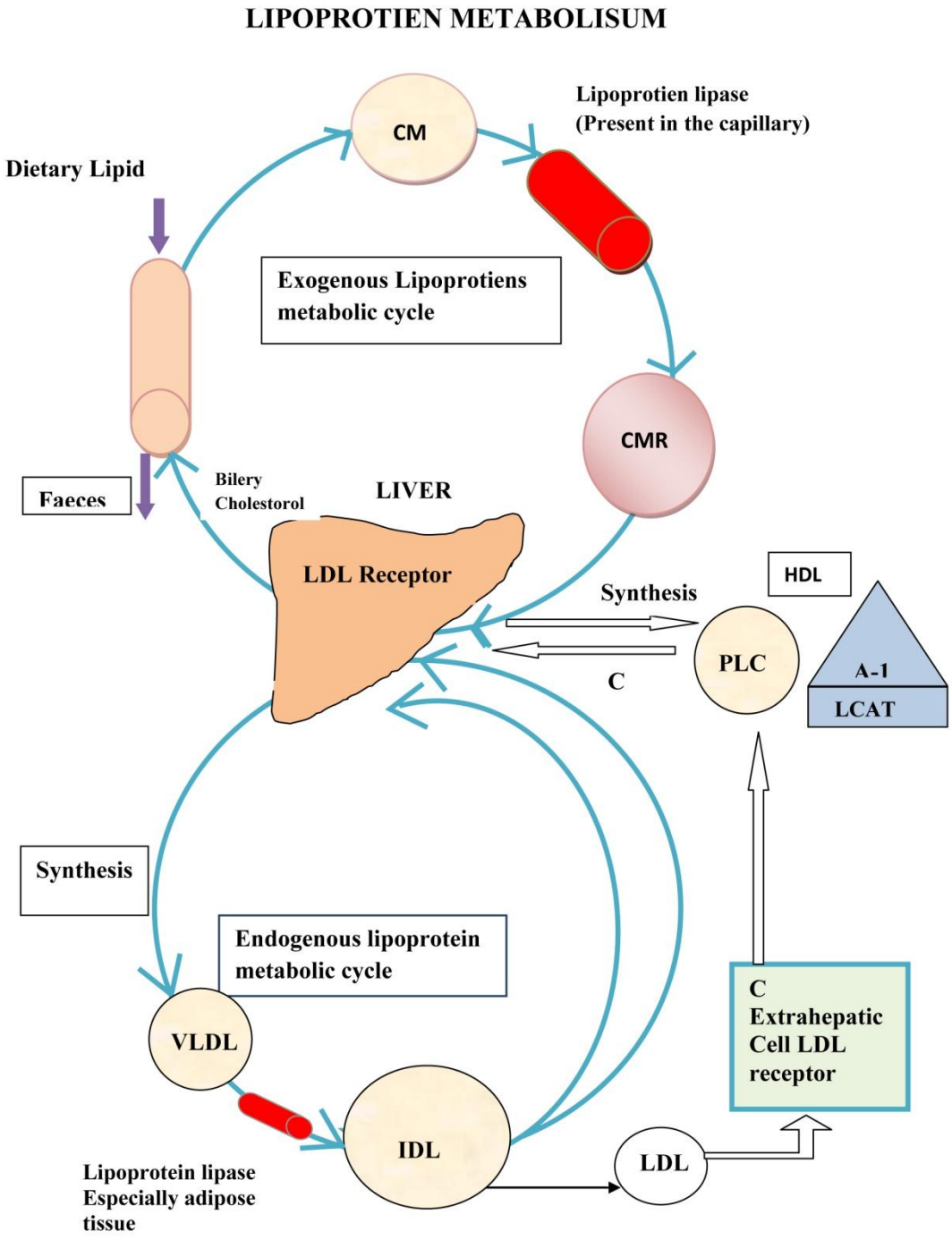
Pathway for Lipid transport:

It comprises two cycles; one exogenous and other endogenous. Both are centered on the liver and interconnected[7].

a) Exogenous pathway: Dietary lipid is absorbed in the small intestine and incorporated into chylomicrons which are secreted into the lymphatics and reach the blood stream via the thoracic duct. Triglycerol is removed from chylomicrons by lipoprotein lipase and tissues absorb FFA and Glycerol. After losing TGs it gets convert into chylomicron remanant which enrich in cholesterol and cholesterol esters. These remanants are removed by the liver where they are catabolised. In the liver cholesterol may be used to form cell membrane or bile salts or may be excreted in the bile. It enters into endogenous pathway[11].

b) Endogenous pathway: The endogenous pathway comprises transport of cholesterol and TGs from liver to muscle, adipose tissue in the form of VLDL. The lipoprotein particles

become smaller but they increase in density to IDL cholesterol and ultimately LDL particles. Cells take up LDL by endocytosis through LDL receptors. Tissue receives cholesterol in the form of HDL particles. After esterification of cholesterol, they are transferred to VLDL or LDL[11].



Atherosclerosis:

Atherosclerosis, a chronic inflammatory disease of the arterial wall, is the major cause of morbidity and mortality from CVD (Cardiovascular Disease). The disease involves the formation of plaques in arterial walls that narrow the arterial passage, obstructing blood circulation and increasing the risk of occlusion of blood flow by a myocardial infarction. Recent information is that atherosclerosis shows a state of heightened oxidative stress characterized by lipid and protein oxidation in the vascular wall[12].

Role of lipids in atherogenic process:

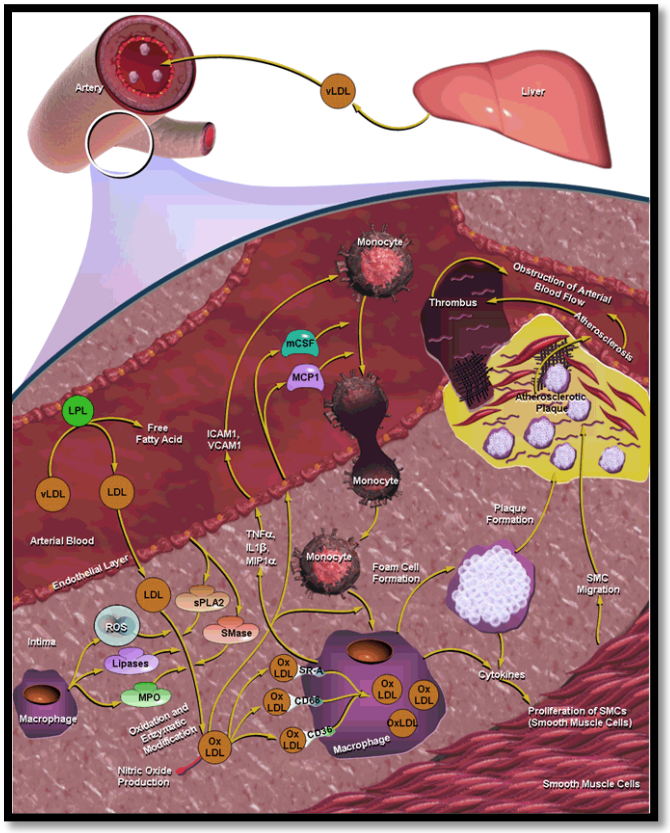
LDLs are the major cholesterol transporters and consist of a hydrophobic core containing cholesteryl ester molecules i.e. Triacylglycerols; a surface monolayer of polar lipids (primarily Phospholipids) and ApoB (Apolipoprotein-B)[13]. LDL in the plasma originates from VLDL (very-Low Density Lipoprotein) produced by the liver. VLDL is transformed to LDL by the action of LPL (Lipoprotein Lipase), an enzyme which hydrolyzes triglycerides in VLDL, The removal of triglycerides from VLDL by LPL leaves a greater proportion of cholesterol, enhancing the density of the particle and changing it to LDL. One of the first steps in the development of atherosclerosis is the passage of LDL out of the arterial lumen into the arterial wall. Plasma LDL is transported across the intact endothelium and caught in the ECM (Extracellular Matrix) of the subendothelial space where it is subjected to oxidative modifications to produce highly oxidized and aggregated LDL, referred to as OxLDL (Oxidized LDL).

Pathogenesis of atherosclerosis:

OxLDL stimulates inflammatory signaling by endothelial cells, releasing chemotactic proteins such as MCP1 (Monocyte Chemotactic Protein-1) and growth factors such as mCSF (Monocyte

Colony Stimulating Factor), which help in the recruitment of monocytes into the arterial wall[13]. OxLDLs also promote the differentiation of monocytes into macrophages that take-up the oxidized LDL in a process that converts them into foam cells. It is the hallmark cell of atherosclerosis. Apart from that, OxLDL also has other effects, such as inhibiting the production of NO (Nitric Oxide), an important mediator of vasodilation and expression of endothelial leukocyte adhesion molecules. The macrophages take up the OxLDLs, become enlarged and full of lipid. These cells accumulate in tissue and are transformed into lipid-laden Foam cells, dying and forming part of the atherogenic plaque (Atherosclerotic Plaque) in the 8fatty streak lesions[14]. Activated macrophages express a range of cytokines (such as TNF-Alpha (Tumor Necrosis Factor-Alpha), IL-1Beta (Interleukin-1Beta), MIP1Alpha (Macrophage Inflammatory Protein-1Alpha) etc.), which stimulate endothelial cells to express adhesion proteins (like VCAM1 (Vascular-Cell-Adhesion Molecule-1), ICAM1 (Intracellular-Adhesion Molecule-1) etc).This facilitates the process of binding of additional blood monocytes to the endothelium and their recruitment into the intima. The cytokines released from the macrophages and foam cells also stimulate the SMCs to migrate into the intima, then proliferate and secrete collagen, elastin and proteoglycans to form a fibrous matrix. This results in the formation of plaques with fibrous caps. The mature plaques protrude into the arterial lumen, and cause obstruction of arterial blood flow. Numerous physiologic triggers: Physical exertion, mechanical stress due to an increase in cardiac contractility, pulse rate, blood pressure and possibly, vasoconstriction initiate the rupture of a vulnerable plaque. Rupture leads to the activation, adhesion, and aggregation of platelets and the activation of the clotting cascade, resulting in the formation of an occlusive thrombus (Clot). Thrombus formation in the lumen of a coronary artery may lead to its partial blockage of blood flow, or, can result in myocardial infarction[13&15].

Cross-sectional view of an artery depicting steps in development of an atheroma, from left to right.



NEED FOR NOVEL NATURAL HYPOLIPIDEMIC AGENT:

Presently, five major classes of medications have been mentioned to treat people with detrimental lipid levels that include statins, nicotinic acid derivatives, fibric acid derivatives, bile acid binding resins and cholesterol absorption inhibitors[6]. However, statins have been associated with most common side effects like stomach upset, nausea, vomiting, headache and dizziness. The common side effects of niacin drugs include flushing, hot flashes, itching and headache[6].

As above mentioned therapy is considered as long term treatment, there may be risk of chronic effects like muscle toxicity, carcinogenic and mutagenic. Hence it is of the hour to explore natural source of medicines those are less toxic, less expensive which can provide better safety and efficacy on a long term usage. Ayurvedic system of medicine consists of various

herbal drugs. Many herbal drugs have been reported to be useful in the treatment of cardiovascular diseases including hyperlipidemea. Among these herbal drugs *Terminalia Arjuna* (Arjuna) and *Emblica Officinalis (Amla)* occupy the pride of place in the context of such medicinal values. Recently there has been renewed interest in these plants because of its multimode cardioprotective activity.

Plant profile of *Terminalia Arjuna* (Arjuna) and biological activities:

Title of plant: *Terminalia Arjuna* (Arjuna)

Classification:

Kingdom- Plantae, Division- Magnoliophyta,
Class- Magnoliopsida, Order- Myrtales,
Family- Combretacae, Gene- Terminalia

Regional names:

English names- Arjuna, Myroblan, White Marudah, white Murdh
Hindi name- Arjan, Arjun, Arjuna, Kahua, Kahu, Koha
Sanskrit name-Arjuna Kakubha, Indradu viravriksha
Latin name- Terminalia Arjuna. **Kannada name-** Maddi, Tora Matti
Gujarati name- Arjun sadada, Sadado. **Telgu name-** Tellamaddi, Yerra maddi
Marathi name- Arjun Sadada, Sadura

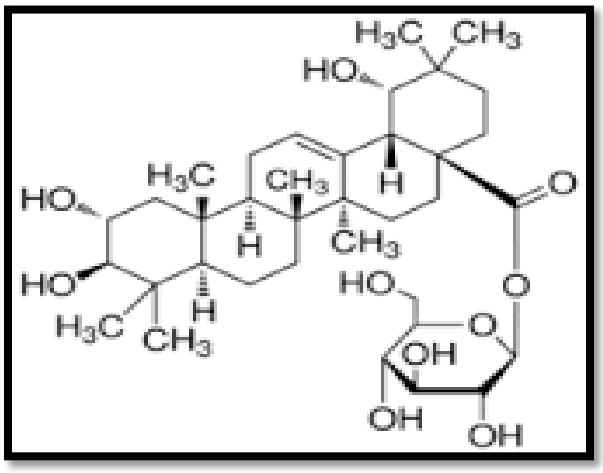
Terminalia Arjuna leaf and fruit

Terminalia Arjuna Tree and Bark

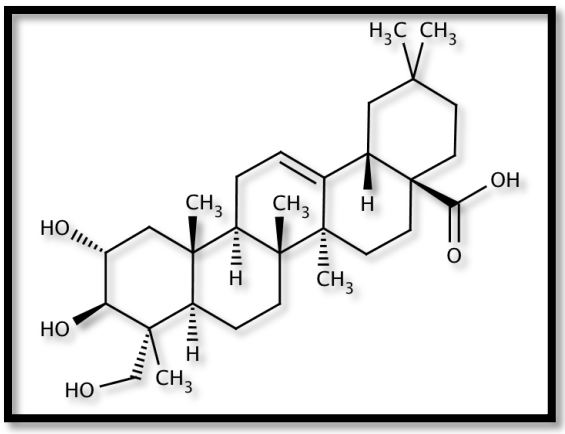
Botanical Details: *Terminalia Arjuna* (Arjuna) is a deciduous tree found throughout India. The Arjuna is about 20–25 metres tall; usually has a buttressed trunk, and forms a wide canopy at the crown, from which branches drop downwards. It has oblong, conical leaves which are green on the top and brown below; smooth, grey bark; it has pale yellow flowers which appear between March and June; its glabrous, 2.5 to 5 cm fibrous woody fruit, divided into five wings, appears between september and november. It has huge, often buttressed trunk and horizontally spreading branches. Extent of buttressing in different localities has been found to be due to local factors and is not determined genetically. Among different species of *Terminalia* the bark of *Terminalia Arjuna* (Arjuna) has its own characteristic features. Bark of *Terminalia Arjuna* (Arjuna) is smooth, pinkish grey from outside and flakes off in large, curved and rather flat pieces. The size of each piece may vary up to 15 cm or more in length, 10 cm in width and 3–10mm in thickness. Sapwood is reddish white and heartwood is brown and variegated with dark colored streaks[16&17].

The histology of *Terminalia Arjuna* (Arjuna) bark reveals the presence of single layered epidermis with hair like projections and few scattered lenticels. Underlying the epidermis is a thin layer of cortex. Periderm and secondary phloem are present in the old bark. Leaves are simple, borne sub-opposite coriaceous, often crenulating, oblong or elliptic. Their upper face is pale or dark green and the lower face is pale brown. It measures 10–15 cm long and 4–7 cm broad. A network of 10–15 pairs of nerves is arranged in reticulate fashion. Petioles are 6–10mm long with yellowish or reddish hairs. Linear, lanceolate-like bracteoles are present. Calyx is glabrous. Its fruit is a drupe, 2.5–5 cm long, ovoid or oblong, fibrous woody, smooth-skinned with five hard angles or wings. The lines of the wings are oblique and curved upwards[16,17&18].

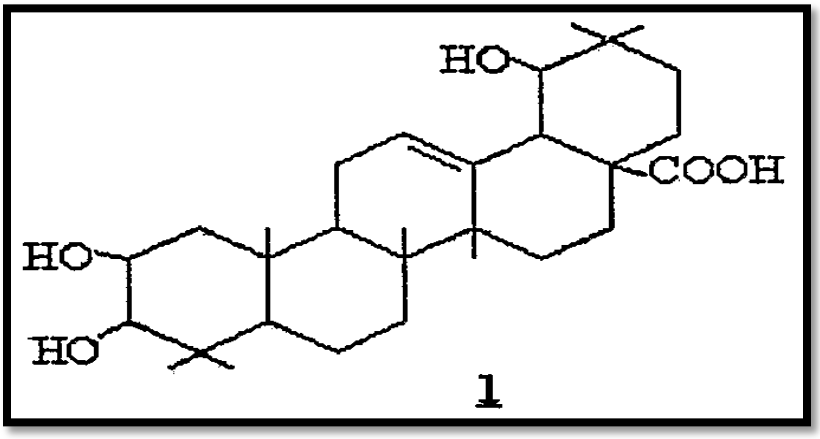
Arjunetin:



Arjunolic Acid:



Arjunolic Acid:



Ethno medical uses:

Arjuna Bark is used as astringent and diuretic[16]. It is used in the treatment of leucorrhea, diabetes and fracture. It is also styptic, antidysentric and expectorant[15]. Chakradatta, the great ancient physician, recommended it to be given as a decoction of bark with milk or as a ghrita (a preparation with ghee or butter)[19]. Decoction of the bark has been used for ulcer cleaning and bark ashes have been recommended for snakebite and scorpion sting. Externally leaves are utilized for applying wounds and sores[20]. It is also used as cardiac tonic and hypolipidemic[21].

Distribution and habitat:

The Arjuna is commonly found growing on river banks or near dry river beds in West Bengal and south and central India. It is found in aplenty throughout Indo-sub-himalayan tracts of Uttar pradesh, south Bihar, Madhya pradesh, Delhi and deccan region near ponds and rivers. It is also found in forests of Sri lanka, Burma and Mauritius[18].

Chemical composition:

Terminalia Arjuna (Arjuna) as a medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids,flavonoids and phenols[21]. Terminalia's active constituents include tannins, cardenolide, triterpenoid saponins (arjunic acid (AA), arjunolic acid, arjungenin (AG), arjun glycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins, phytosterols, calcium, magnesium, zinc and copper[22&23].

Plant profile of *Emblica Officinalis (Amla)* and its biological activities:

Title of plant: *Embilica Officinalis (Amla)*

Classification:

Kingdom- Plantae

Division- Angiospermae

Class- Dicotyledonae

Order- Geraniales

Family- Euphorbiaceae

Gene- Emblica

Species: *Officinalis* geartn**.**

Regional names:

English: Emblic myrobalan, Indian goose berry

Sanskrit: Aamalaki, **Hindi:** Amla, **Kannada:** Nelli Kayi, **Marathi:** Amla, **Gujarati:** Ambla,

Malayalam: Nelli Kayi, **Tamil**: Nelli, **Telugu:** Usirikaya and **Kashmir:** Aonla.

Emblica Officinalis (Amla) Tree

Emblica Officinalis (Amla) fruit

BOTANICAL DETAILS:

Amla is a small to medium sized deciduous tree, reaching 8 to 18 in height, which is known for its edible fruit of the same name. The tree has crooked trunk and spreading branches. The leaves are simple, nearly stalk less and closely set along slender branchlets. The leaves are often mistaken for leaflets of pinnate leaves. The genus name phyllanthus is derived greek words meaning leaf flower, an allusion to the apparent bearing of flowers on the leaves. Amla flowers small, greenish- yellow or pinkish. The flowers have six segments, but no real petals. Male and female flowers are carried separately on the same branch. The fruit is nearly spherical, light greenish yellow, quite smooth and hard on appearance, with 6 vertical strips or furrows. Ripening in autumn, the berries are harvested by hand after climbing to upper branches bearing the fruits. The taste of Amla is sour, bitter and astringent, and is quite fibrous[24].

Distribution and habitat: *Emblica Officinalis (Amla)* is an evergreen tree which is highly prized in tropical Asia. Found throughout India, the sea-coast districts and on hill slopes up to 200 meters, also cultivated in plains. It is common all over tropical and sub-tropical India and also found in Burma. It is abundant in deciduous forests of Madhya pradesh, the deccan, the sea-coast districts and Kashmir. Grows in tropical and subtropical parts of Ceylon, Malay Peninsula, Pakistan, Bangaladesh, Shrilanka and China. In Ceylon, it is very common in exposed places on patana land in the moist regions up to 4000 feet altitude[25].

Chemical composition:

Emblica Officinalis (Amla) primarily contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C (478.56 mg/100 mL). Compounds isolated from *Emblica officinalis (Amla)* were gallic acid, ellagic acid, 1-O-galloyl-beta- D-glucose, 3,6-di-O-galloyl-Dglucose, chebulinic acid, quercetin, chebulagic acid,

corilagin, 1,6-di-O - galloyl beta D glucose, 3 Ethylgallic acid (3 ethoxy 4,5 dihydroxy benzoic acid) and isostrictiniin. It also contains flavonoids, kaempferol 3 O alpha L (6'' methyl) rhamnopyranoside and kaempferol 3 O alpha L (6''ethyl) rhamnopyranoside. A new acylated apigenin glucoside (apigenin 7 O (6'' butyryl beta glucopyranoside) was isolated from the methanolic extract of the leaves of Phyllanthus emblica together with the known compounds; gallic acid, methyl gallate, 1,2,3,4,6-penta-Ogalloylglucose and luteolin-4'-Oneohesperiodoside were also reported[26].

Ethno medical uses:

Emblica enjoys a hallowed position in Ayurveda, an indigenous system of medicine in India[27]. It is proved to be effective against diabetes, cough, asthma, bronchitis, dyspepsia, colitis, hyper acidity, peptic ulcer, skin diseases, inflammations, anemia, hepatopathy, jaundice, diarrhoea, dysentery, haemorrhage, leucorrhoea, cardiac disorders, intermittent fevers and greying of hair and is given to cancer. The plant is known for digestion power, improving liver functions and is liver protective. It is also a very good blood purifier which in turn improves the health of liver by keeping the toxins and infections away. It is also used as antioxidant, cardioprotective, strengthen heart and hypolipidemic[28].

***Terminalia Arjuna (Arjuna)*:**

1) **Cardioprotective effect:**

The cardinal benefits of *Terminalia Arjuna (Arjuna)* are enhancement of cardiac muscle function and subsequent improved pumping activity of the heart. Studies reported that the saponin glycosides could be responsible for the inotropic effects of *Terminalia*, where as the flavonoids and OPCs spare free radical antioxidant activity and vascular strengthening[29&30].

Kumar et al reported in their study that *Arjuna* protects the heart by beta receptor stimulation against myocardial changes[31].

2) **Hypolipidemic and antiatherogenic activity:**

Animal studies suggest *Terminalia* might reduce blood lipids. Rats made hyperlipidemic by feeding them an atherogenic diet were given an oral ethanolic extract *Terminalia*. Animals given *Terminalia* had a significant, dose-related decrease in levels of LDL-cholesterol and TGs compared to rats received atovastatin[32].

Khanna A K reported in their study that rats fed cholesterol (25 mg/kg body weight) alone or along with *Terminalia* bark powder (100 mg/kg) for 30 days, *Terminalia* feeding caused a smaller increase in blood lipids and an increase in HDL cholesterol compared to the cholesterol-only group. The researchers felt inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid excretion, and stimulation of receptor mediated catabolism of LDL cholesterol caused *Terminalia's* lipid-lowering effects[33].

3) **Endothelial Dysfunction:**

Hydroalcoholic extract of bark of *Terminalia Arjuna (Arjuna)* has shown marked regression of endothelial abnormality amongst smokers compared to age matched non smokers[34].

4) Hepatoprotective:

The literature survey reported that the ethanolic extract of *Terminalia Arjuna (Arjuna)* bark are found to be used in the traditional system of medicine as a liver tonic. *Terminalia Arjuna (Arjuna)* is widely used in the treatment of liver diseases like hepatitis, cirrhosis, and loss of appetite. Arjuna is best hepatitis reliever. Leaves of *Terminalia Arjuna (Arjuna)* are best healing for hepatitis[18].

***Emblica Officinalis (Amla)*: Amla as antioxidant:**

Oxidative stress is occurring mainly due to imbalance between pro-oxidant and antioxidant homeaostatic phenomenon in the body. Pro-oxidant increase in the body due to generation of free radicals or poor scavenging in the body. Free radicals are mainly produced during normal respiration and by cell mediated immune functions. Any overburden of free radicals leads to decrease in antioxidant. This may result in damage to tissues and subsequent diseases[26].

Amla as has been shown to possess good antioxidant property. Evaluation of *Emblica Officinalis* (Amla) in rodents has proved it to be ameliorative against increased lipid peroxidation as well as a decreased activity of enzymatic antioxidants and non-enzymatic antioxidants in both the organs[35].

Cardioprotective Activity of *Emblica Officinalis (Amla)*:

It has shown an increase in the cardiac glycogen suggesting a cardio protective action. *Emblica Officinalis (Amla)* has shown its role against isoproterenol-induced cardiotoxicity showed cardioprotective potential along with antioxidant activity and favourable improvement in hemodynamic and contractile function[36].

Chronic *Emblica Officinalis (Amla)* administration produces myocardial adaptation by augmenting endogenous antioxidants and protects rat hearts from oxidative stress associated with ischaemic reperfusion injury[37].

Role of Emblica Officinalis in Hyperlipidemia:

Many experiments on animals demonstrated improved lipid profile and reduced hypertension in induced metabolic syndrome in rats[38]. Flavonoids extract from the fruits of Amla inhibited synthesis and increased degradation of cholesterol via increased hepatic HMG-CoA reductase[38]. Cu(2+)-induced LDL oxidation and cholesterol-fed rats were used to examine the effects of amla

on low-density lipoprotein (LDL) oxidation and cholesterol levels *in vitro* and *in vivo*. It was concluded that Amla can be effective for hypercholesterolemia and prevention of atherosclerosis[39].

Hepatoprotective Effect:

Emblica Officinalis (Amla) acts as hepatoprotective in various liver disorders. Arsenic exposure in mice also caused a significant change in serum biomarkers in the SGOT, SGPT and creatinine as compared to the controls. There were no significant changes in the serum levels of total protein in these mice. Co-administration of arsenicand fruit extract of amla (500 mg/kg body weight/day for 30 days) caused a significant reduction of arsenic transference associated with significantly decreases hepatic arsenic levels and balanced the antioxidant enzyme and levels of serum hepatic enzymes like SGOT and SGPT. This clearly demonstrates the antioxidant property of amla that could be responsible for its protective efficacy in arsenic induced hepatic toxicity[40].

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CHAPTER 4

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS: Albino Wistar rats weighing 180 to 250gms were obtained from animal house of Shri B M Patil Medical college Hospital & Research Centre, BLDE University, Bijapur. All the five group animals were acclimatized for 7 days to the laboratory conditions at 22-24^{0}C and maintained 12 HR. Light/dark cycle. All the experimental procedures were performed in accordance with the approval of the Institutional Animal Ethics Committee (IAEC) of Shri B M Patil Medical college Hospital & Research Centre, Bijapur.

All the care has been taken on animals during experimental as well as at the time scarification as per the guidelines of ICMR (Indian Council of Medical Research) on animal research 2006. An experiment was performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), INDIA. After completion of the experimental protocol, animals were sacrificed by cervical dislocation and incinerated electrically[1].

AUTHENTICATION OF DRUGS:

Fresh fruits of *Emblica Officinalis (Amla)* and Bark of *Terminalia Arjuna* were collected from the local market in Bijapur and obtained the botanical authentication of these drugs in the Dept of Botany, KCP Science College, Bijapur.

PERMISSION EXTRACTION OF DRUGS:

Obtained permission for ethanolic extraction of *Emblica Officinalis (Amla)* and *Terminalia* (Arjuna) drugs from the Department of Pharmacology BLDEA College of Pharmacy Bijapur

EXTRACTION OF DRUG:

Ethanolic extract preparation: Powder of dry bark of *Terminalia Arjuna* and powder of dry fruits of *Emblica Officinalis (Amla)* was extracted with 99% ethanol using a soxhlet

apparatus at a temperature below 60° C for 22 hours. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder.

Percent of Yield was calculated as follows:

$$\text{Extract yield \%} = \frac{W1}{W2} \; 100$$

Where, W1= Net weight of powder in grams after extraction and W2= Total weight of wood powder in grams taken for extraction[2].

Extraction of drugs by Soxhlet apparatus

Phytochemical Screening

Different extracts were screened for the presence of phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate by using standard protocols[3].

Phytochemical Investigations:

Chemical and reagents: Laboratory grade chemicals were used for routine work. Analytical grade reagents (A.R.) were used for analytical work

Absorbents: Silica gel GF254 (RFCL Ltd Renkem production, New Delhi) was used for TLC

Preliminary Phytochemical studies: The crude extract obtained by extraction from ethanolic extraction was subjected phytochemical studies

A. Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

1.Molisch"s test: To 1 ml of test solution added a few drops of 1 % alpha-napthol and 2-3 ml concentrated sulfuric acid along the side of test tube. The reddish violet or purple ring formed at the junction of two liquids confirmed the test.

2. Benedict's test: Filtrates were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.

3.Fehlings test: Dissolved 2 mg dry extract in 1 ml of distilled water and added 1ml of Fehling's (A+B) solution, shooked and heated on a water bath for 10 minutes. The brick red precipitate formed confirmed the test

B. Detection of fixed oils & fats

1. Stain Test: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oil.

C. Detection of phytosterols

1. Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

2. Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicates the presence of phytosterols.

D. Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

1. Modified Borntrager's Test: Extracts were treated with a ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

E. Detection of flavonoids

1. Alkaline Reagent Test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
2. Lead acetate Test: Extracts were treated with a few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.
3. Shinoda Test: To the alcoholic solution of extracts, a few fragments of magnesium ribbon and Conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids. Alcoholic solution of extracts, a pinch of zinc dust and conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

F. Detection of alkaloids

Approximately 50 mg of extract was dissolved in 5 ml of distilled water. Further 2M hydrochloric acid was added until an acid reaction occurred and filtered. The filtrate was tested for the presence of alkaloids as detailed below. Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

1. Mayer's Test: Filtrates were treated with mayer's reagent (Potassium Mercuric iodide). Formation of a yellow, cream precipitate indicates the presence of alkaloids.

2. Wagner's Test: Filtrates were treated with wagner's reagent (Iodine in potassium iodide) Formation of brown/reddish brown precipitate indicates the presence of alkaloids.
3. Dragendorff's Test: To 2 ml of the filtrate was added 1 ml of dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the test as positive.
4. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

G. Detection of saponins

1. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
2. Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins.
3. Olive oil test: - Added a few drops of olive oil to 2ml of the test solution and shaken well. The formation of a soluble emulsion confirmed the test.

H. Detection of resins

1. Acetone-water Test: Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

I. Detection of phenols

1. Ferric Chloride Test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

J. Detection of tannins

1. Ferric chloride Test: Added a few drops of 5% ferric chloride solution to 2 ml of the test solution. Formation of blue colour indicated the presence of hydrolysable tannins.

2. Gelatin Test: Added five drops of 1% gelatin containing 10% sodium chloride to 1 ml of the test solution. Formation of white precipitates confirmed the test.

STUDY DESIGN

Experimental protocol: All the rats were divided into following five groups with 6 rats in each group. Group-I, fed Iso-caloric diet and serve as a control, the Group II Fed with high fat diet, Group-III fed with high fat diet with ethanolic extract of *Terminalia Arjuna (Arjuna),* Group IV fed with high fat diet with ethanolic extract of *Emblica Officinalis (Amla)* and Group V fed with high fat diet with ethonolic extract of *Terminalia Arjuna and Emblica Officinalis (Amla).* It was given daily *Terminalia Arjuna (Arjuna)* 500mg/kg B^2. wt and *Emblica Officinalis(Amla)* 100mg/kg B.Wt, I.P. for three weeks[4].

Rats Group	**Normal & Food Hyperlipidemic**	**Dosages *Terminalia Arjuna (Arjuna)***	**Dosages *Emblica Officinalis (Amla)***
Group1. Normal diet	a. Carbohydrate 62%. b. Protein 18%. c. Fat 20%. d. Salt 4%. e. Multi vitamins 1%	--	--
Group 2. Hyperlipidemic diet	a. Carbohydrate 52%. b. Protein 18%. c. Fat 30%. d. Salt 4%. e. Multi vitamins 1%	--	--
Group 3. Hyperlipidemic diet Treated with *Terminalia Arjuna (Arjuna)*	a. Carbohydrate 52%. b. Protein 18%. c. Fat 30%. d. Salt 4%. e. Multi vitamins 1%	100mg/200gm of Rats. For 3 weeks	--
Group 4. Hyperlipidemic diet Treated with *Emblica Officinalis (Amla)*	a. Carbohydrate 52%. b. Protein 18%. c. Fat 30%. d. Salt 4%. e. Multi vitamins 1%	--	100mg/Kg For 3 weeks
Group 5. Hyperlipidemic diet Treated with *Terminalia Arjuna(Arjuna)* and *Emblica Officinalis(Amla)*	a. Carbohydrate 52%. b. Protein 18%. c. Fat 30%. d. Salt 4%. e. Multi vitamins 1%	100mg/200gm of Rats. For 3 weeks	100mg/Kg For 3 weeks

PREPARATION OF ISO-CALORIC AND HYPERLIPIDEMIC DIET

PREPARATION OF ISO-CALORIC DIET:

Normal diet (Group 1) 1kg of Iso-caloric diet contains 620gms of carbohydrate, 180gms of protein, 200gms of fat, 2gms of Na+ and 1% of multivitamins.

In this group each rat fed with Iso-caloric diet per day on and average 50gms. We observed that all rats consumed not more than 40gms. Each rat consumed 35gms instead of 40gms, the left over 5gms of food discarded and next day fresh 40gms of food was served[5].

PREPARATION OF HYPERLIPIDEMIC DIET:

Hyperlipidemic diet (Group 2 to 5) 1kg of hyperlipidemic diet contains 520gms of carbohydrate, 180gms of protein, 300gms of fat, 2gms of Na+ and 1% of multivitamins.

In these groups, each rat fed with hyperlipidemic diet per day on and average 50gms. We observed that few of rats consumed more than 45gms of diet and a few of them consumed less than 40gms of diet[6].

GENERAL

Sample collection

Every alternate week (six rats) one group of animals was sacrificed by cervical dislocation at the end of the last dose with an overnight fast. Blood was collected in normal tubes for the separation of serum, by doing retro-orbital puncture, before sacrificing the animals

Cervical dislocation method

1. Restrain the rodent in a normal standing position on a firm, flat surface and grasp the base of the tail firmly with one hand. Performing the procedure on a surface that the animal can grip (such as the wire bar grid of the cage top) may make it easier to gain access to the base of the skull because rodents often stretch themselves forward when held by the tail.
2. Place a sturdy stick-type pen, a rod-shaped piece of metal, closed scissors/hemostats or the thumb and first finger of the other hand against the back of the neck at the base of the skull.
3. To produce the dislocation, quickly push forward and down with the hand or object restraining the head while pulling backward with the hand holding the tail base.
4. The effectiveness of dislocation can be verified by feeling for a separation of cervical tissues. When the spinal cord is severed, a 2-4 mm space will be palpable between the occipital condyles and the first cervical vertebra. Occasionally, however, the dislocation occurs between thoracic vertebrae.
5. Check closely to confirm respiratory arrest, and when possible verify, by palpation, that there is no heart beat[7].

HAEMATOLOGY

After the experimental period blood was collected from the rats by retro orbital venous puncture and blood was collected in heparinized tubes and non heparinized tubes.

Haematological parameters were evaluated by XS-1000i closed tube sampling sysmax automated analyzer (Calibrated by Sysmex division of MYCO Instrumentation Ltd).

Measurement principle:

RBC/PLT- sheath flow direct current on XE and XT series. WBC-semicoductor laser flow cytometry, HGB-clorometric method (SLS).

Testing parameters CBC and S-Part differential 21 parameters like Hb% RBC WBC Platelet PCV & MCHC were analyzed[8].

LIPID PROFILE

Serum triglycerides (TG), Serum total cholesterol (TC), High-density lipoprotein (HDL), Low-density lipoprotein (LDL) and Very Low-density lipoprotein (VLDL) were analysed.

i) Total cholesterol

Principle: Enzymatic determination of total cholesterol was performed according to the following equation

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{Fatty acids},$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{4- Cholesten- 3- one} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4- aminoantipyrene} \rightarrow \text{Red quinine} + 4H_2O$$

Cholesterol reagent

Pipes buffer, pH 6.7- 50 mmol/l

Phenol- 24 mmol/l

Sodium cholate- 0.5 mmol/l

4- aminoantipyrene- 0.5 mmol/l

Cholesterol esterase > 180U/l

Cholesterol oxidase > 200U/l

Peroxidase > 1000U/l

Cholesterol standard solution 200 mg/dl

The reagent is stable for 18 months when stored at 2-8° C. Animal serum was used as the sample. 10 µl of serum was mixed with 1000 µl of reagent, incubated for 5 min at 37 °C and estimated at 630 nm using a Biochemical Analyzer.

Calculation

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of sample x 200}}{\text{Absorbance of standard}}$$

ii) Triglycerides

Principle: Enzymatic determination of triglycerides was performed according to the following equation[9].

Triglyceride + H2O ⟹ Glycerol + Fatty acids

Glycerol + ATP ⟹ glycerol-3- phosphate + ADP

Glycerol-3-PO4 + O2 ⟹ dihydroxy acetone phosphate+ H2O2

H2O2 + 4- aminoantipyrine + p- chlorophenol ⟹ Red quinoneimine

Reagent Composition

Pipes buffer, pH 7.0- 50 mmol/l

p-chlorophenol- 5.3 mmol/l

Potassium ferrocyanate- 10 mmol/l

Magnesium salt- 17 mmol/l

4- aminoantipyrine- 0.9 mmol/l

ATP- 3.15 mmol/l

Lipoprotein lipase > 1800 U/l

Glycerol kinase > 450 U/l

Glycerol-3- phosphate oxidase > 3500 U/l

Peroxidase > 450 U/l

Triglyceride standard solution- 200 mg/dl

The reagent was stable for 18 months when stored at 2-8° C. Animal serum was used as the sample. 10 µl of serum was mixed with 1000 µl of reagent, incubated for 5 min at 37 ° C and estimated at 630 nm using a biochemical analyzer.

Calculation:

$$\text{Triglyceride (mg/dl)} = \frac{\text{Absorbance of sample} \quad \text{x } 200}{\text{Absorbance of standard}}$$

iii) HDL cholesterol

Principle: The chylomicrons, VLDL and LDL of serum were precipitated by phosphotungstic acid and magnesium ions. After centrifugation, HDL in the supernatant solution was measured by enzymatic method[10].

HDL cholesterol reagent

Phosphotungstate- 14 mmol/l

Magnesium chloride- 1 mmol/l

Preservative

HDL cholesterol standard- 50 mg/dl.

Animal serum was used as the sample. 300 µl of serum was mixed with 300µl of HDL reagent, allowed to stand for 10 min at room temperature, mixed again and centrifuged for 10 min at 4000 rpm. After centrifugation the clear supernatant was separated from the precipitate within 1hr and HDL was determined using cholesterol reagent. 50 µl of supernatant was mixed with 1000 µl of cholesterol reagent, incubated for 5 min at 37 ° C and estimated at 630nm using a biochemical analyzer.

Calculation

$$\text{HDL cholesterol conc. (mg/dl)} = \frac{\text{Absorbance of sample x N x 2}}{\text{Absorbance of standard}}$$

N= Standard concentration (50 mg/dl)

iv) Estimation of LDL

Formula: LDL = TC/1.19 + TG/1.9 – HDL/1.1 – 38 (mg/dl)[11].

v) Estimation of VLDL

Formula

$$\text{VLDL} = \frac{\text{triglycerides}}{5} \text{ (mg/dl)}^{11}.$$

1. LIVER FUNCTION TEST:

1 **Assessment of liver function test**

Serum was separated by centrifuging blood at 2500 rpm for 10 minutes and the levels of SGOT, SGPT, bilirubin, ALP, albumin, A/G ratio and total protein were analyzed by using a commercially available enzymatic kit (AGAPPE, India) and an autoanalyser (Chemistry Analyser (CA 2005), B4B Diagnostic Division, China).

i) Estimation of Serum Glutamate Pyruvate Transaminases (SGPT/ ALT)

Principle: Alanine aminotransferase catalyses the transfer of amino group from alanine to 2-oxoglutarate, resulting in the formation of pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm, by means of lactate dehydrogenase coupled reaction[12].

The enzymatic reaction employed in the assay of SGPT is as follows.

$$\text{L- Alanine + 2-oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + L- Glutamate}$$

$$\text{Pyruvate + NADH+ H}^{+} \xrightarrow{\text{LDH}} \text{D- Lactate + NAD}^{+}$$

Reagent Preparation: Reagent A: Tris 150 mmol/l, L- Alanine 750 mmol/l, lactate dehydrogenase >1350U/l, pH 7.3. **Reagent B:** NADH 1.3 mmol/l, 2-oxoglutarate 75 mmol/l, sodium hydroxide 148 mmol/l, sodium azide 9.5 g/l. Auxillary Reagent **Reagent C:**

Pyridoxal phosphate 10 mmol/l. Working Reagent: Reagent A (4 parts) is mixed with 1 part of Reagent B. The combined reagent was stable for 2 months at 2-8° C. The mixed reagent was stored in a dark place and protected from light. Working Reagent with Pyridoxal phosphate: 10ml of working reagent was mixed with 0.1 ml of reagent C. The solution was stable for 6 days at 2-8° C.

Procedure: Animal serum was used as the sample. 50 µl of serum was mixed with 1000 µl of mixed reagent and estimated in kinetic mode using a Biochemical Analyzer.

Calculations

$$\text{SGPT/ ALT concentration (U/l)} = \frac{\text{delta A/min x Vt x } 10^6}{\text{E x L x Vs}}$$

Molar absorbance (E) of NADH at 340 nm is 6300

L - Light path 1cm

Vt - Total reaction volume is 1.05 at 37° C

Vs - Sample volume is 0.05 at 37° C

ii) Estimation of Serum Glutamate Oxaloacetate Transaminases (SGOT/ AST)

Principle: Aspartate aminotransferase catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm, by means of malate dehydrogenase (MDH) coupled reaction[12].

The enzymatic reaction employed in the assay of SGOT is as follows.

2-oxoglutarate Glutamate + Oxaloacetate

Oxaloacetate + NADH+ H+ D-Malate + NAD+

Reagent Preparation: Reagent A: Tris 121 mmol/l, L- aspartate 362 mmol/l, malate dehydrogenase >460 U/l, lactate dehydrogenase > 660 U/l, sodium hydroxide 255 mmol/l, pH 7.8. **Reagent B:** NADH 1.3 mmol/l, 2-oxoglutarate 75 mmol/l, sodium hydroxide 148 mmol/l, sodium azide 9.5 g/l.

Auxillary reagent – Reagent C: Pyridoxal phosphate 10 mmol/l.

Working reagent: **Reagent A** (4 parts) is mixed with 1 part of **Reagent B**. The combined reagent is stable for 2 months at 2-8° C. The mixed reagent was stored protected from light. Working reagent with pyridoxal phosphate: 10ml of working reagent was mixed with 0.1 ml of **Reagent C**. Stable for 6 days at 2-8° C. Animal serum was used as the sample.

Procedure: 50 µl of serum was mixed with 1000 µl of mixed reagent and estimated in kinetic mode using a biochemical analyzer.

Calculations:

$$\text{SGOT/ AST concentration (U/l)} = \frac{\text{delta A/min x Vt x } 10^6}{\text{E x L x Vs}}$$

Molar absorbance (E) of NADH at 340nm is 6300

L - Light path 1cm

Vt - Total reaction volume is 1.05 at 37° C

Vs - Sample volume is 0.05 at 37° C

iii) Estimation of Alkaline Phosphatase

Principle: Alkaline Phosphatase catalyses in alkaline medium the transfer of phosphate group from 4-nitrophenyl phosphate to 2-amino-2-methyl-1-propanol, liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405nm[13].

The enzymatic reaction employed in the assay of alkaline phosphatase is as follows.

4- Nitrophenyl phosphate + H_2O $\xrightarrow{\text{ALP}}$ Phosphate + 4- Nitrophenol

Reagents: Reagent A: 2- Amino-2- methyl- 1- propanol 0.4 mol/l

Zinc sulphate 1.2 mmol/l

N hydroxy ethylene diamine tri aceticacid 2.5 mmol/l

Magnesium acetate 2.5 mmol/l, pH 10.4.

Reagent B: 4- Nitrophenyl phosphate 60 mmol/l.

Working reagent: 4 parts of reagent A is mixed with 1 part of reagent B. The combined reagent is stable for 2 months at 2-8° C. Animal serum was used as the sample 20 µl of serum was mixed with 1000 µl of mixed reagent and estimated in kinetic mode using a biochemical analyzer.

Calculations

$$\text{ALP concentration (U/l)} = \frac{\text{delta A/min x Vt X } 10^6}{\text{E X L X Vs}}$$

Molar absorbance (E) of NADH at 405nm is 18450

L- Light path 1cm

Vt - Total reaction volume is 1.02 at 37° C

Vs - Sample volume is 0.02 at 37° C iv)

iv. Total bilirubin

Principle: Direct bilirubin in the sample reacts with diazotised sulfanilic acid forming a coloured complex that can be measured by spectrophotometry. Both direct and indirect bilirubin couple diazo in the presence of cetrimide[14].

The terms direct and total refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilizing reagents. The direct and indirect bilirubin is approximately equivalent to the conjugated and unconjugated fractions.

Composition (Bilirubin)

Reagent A: Sulfanilic acid 29 mmol/l

Hydrochloric acid 0.2 mol/l

Cetrimide 50 mmol/l

Reagent B: Sodium nitrite- 11.6 mmol/l. Stored at 15-30° C. Reagents were stable until the expiry date shown on the label when stored tightly closed and if contaminations was avoided during use. Presence of particulate matter, turbidity, absorbance over 0.05 at 540nm, indicate deterioration.

Working Reagent preparation: Mixture of 1 ml of Reagent B and 4 ml of Reagent A. This was Stable for 20 days at 2-8° C.

Particulars	Reagent Blank	Sample Blank	Sample	Standard
Distilled water	100 µl	--	--	--
Sample	--	100 µl	100 µl	--
Standard	--	--	--	100 µl
Reagent A	--	1000 µl	--	--
Working Reagent	1000 µl	--	1000 µl	1000 µl

Mixed thoroughly and was allowed to stand for 2 min at room temperature. Absorbance of Sample Blank was read at 540nm against distilled water and absorbance of Sample was read at 540nm against reagent blank.

Calculations

$$\text{Bilirubin content in the sample} = \frac{\text{A (sample)} - \text{A (sample blank)}}{\text{A (standard)}} \text{ X C (standard)}$$

Mass concentration (mg/dl) x 17.1 = Substance concentration in µmol/l.

v) Total protein estimation

Principle: The enzymatic reaction sequence employed in the assay of total protein was as follows:

Protein + Cu2+ Cu – Protein complex

Total proteins were estimated using Total protein reagent from AGAPPE Diagnostics, Kerala, India[15].

Composition of Total protein reagent

Potassium iodide- 6 mmol/l. Potassium sodium tartrate- 21 mmol/l. Copper sulphate- 6 mmol/l. Sodium hydroxide- 58 mmol/l. Total Protein Standard- 6 g/dl.

The reagent was stable for 18 months when stored at 2-8° C. To 20 µl of serum, 1ml of total protein reagent was added and mixed. The mixture was incubated at 37oC for 15 minutes and the absorbance was measured at 546 nm using a biochemical analyzer.

The protein content was calculated by using the following formula and expressed as total protein in g/dl.

$$\text{Total protein in g/dl} = \frac{\text{Absorbance of sample x C}}{\text{Absorbance of standard}}$$

Where C refers to the protein concentration in standard protein solution in g/dl.

vi) Albumin estimation

Principle: The reaction between albumin in serum or plasma and the dye bromocresol green produces a change in colour, which is proportional to albumin concentration[16].

Reagent Composition

Albumin reagent: Succinate buffer (pH- 4.2) 75 mmol/l. Bromocresol green 0.14 g/l.

Albumin Standard: Albumin Standard concentration 3 g/dl. The reagent is stable for 18 months when stored at 2-8o C. Animal serum was used as sample. 10 µL of serum was mixed with 1000 µL of reagent, mixed and incubated for 1 minute. The absorbance was measured against blank at 630 nm.

Calculation

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of sample x C}}{\text{Absorbance of standard}}$$

Where C refers to the albumin concentration in standard albumin solution in g/dl

2. SERUM ELECTROLYTE

Serum Na^+ (Sodium), K^+ (Potassium) and Ca^{++} (Calcium) were analysed by Meril Diagnostic Kit Method: Merilyzer Cliniquant easylyte analyzer

3. **GLUCOSE ESTIMATION:** Random blood sugar glucose oxidase method

Principle: The substrate β-D-glucose is oxidized by glucose oxidase to from gluconic acid and hydrogen peroxide. The hydrogen peroxide so generated oxidizes the chromogen system consisting of 4-aminoantipyrine and phenolic compound to a red quinoeimine dye. The intensity of the colour produced is proportional to the glucose concentration and is measured at 505 nm (490-530 nm) or with green filter[17].

Glucose

Glucose + O_2 $\xrightarrow[\text{Oxydase}]{}$ Gluconic acid + H_2O_2

H_2O_2+Phenolic compound+4-Aminoantipyrine $\longrightarrow$ Red Quinoemine+H_2O

Kit contents Reagent1: Glucose reagent. Reagent 2: Glucose standard (For calibration)

Procedure for estimation of glucose

Pipetted into microcentrifuge tubes	Blank (µl)	Standard (µl)	Test (µl)
Glucose Reagent	500	500	500
Calibrator (Standard)	--	5	--
Sample (Serum)	--	--	5
Distilled water	5	--	--

Mixed and incubated at 37oC for 10 minutes. Absorbance of the Test (AT), Standard (AS) and Reagent Blank (AB) at 505 nm was read against distilled water using biochemical analyzer.

Calculations: Glucose (mg/dl) = (AT-AB/AS-AB) x100

Where 100 = Standard concentration of Glucose (mg/dl)[18].

4. **ESTIMATION OF NITRIC OXIDE:** Method: Griess method (Kinetic cadmium reduction)

Nitric oxide was determined as nitrite in serum by a kinetic cadmium reduction method by Najwa cortas and Nabil wakid[19].

Principle: Nitrate the stable product of nitric oxide is reduced to nitrite by cadmium reduction method after deproteinization of sample by somogyi reagent. The nitrite produced is determined by diazotization with sulphanilamide and coupling to Nnaphthylethylenediamine. The intensity of coloured complex is measured at 540 nm.

Reagents:

1. Cadmium granules (2.5 – 3 gram granules in assay, stored in 0.1M/L H_2SO_4)

2. Glycine–NaOH buffer (pH 9.7): 7.5 gm of glycine was dissolved in 200ml deionised water, then the pH was adjusted to 9.7 by 2M NaOH and was diluted to 500 ml by deionised water.

3. Sulfanilamide: 2.5 gm of sulfanilamide was dissolved in 250 ml of warm 3M/L HCl and allowed to cool.

4. N-naphthylethylenediamine : 50 mg of N-naphthylethylenediamine was dissolved in deionised water and the volume was adjusted to 250 ml.

5. Stock standard sodium nitrite solution (0.1 mol/L): 690 mg sodium nitrite was dissolved in 100 ml of 10 mmol/L of sodium borate solution.

6. Working standard solution (10 µmol/L): 10µl of stock was diluted to 100 ml with 10mmol/L solution of sodium borate.

7. $ZnSO_4$ solution (75 mmol/L)

8. NaOH solution (55 mmol/L)

9. H_2SO_4 solution (0.1 mol/L)

10. $CuSO_4$ solution (5 mmol/L): 125 mg of $CuSO_4$ was dissolved in 100 ml of glycine - NaOH buffer.

Procedure: Part I: - Deproteinization: A centrifuge tube was taken and additions were made as follows:

Serum.	0.5 ml
75 mM $ZnSO_4$	2.0 ml
55 mM NaOH	2.5 ml

Tube was centrifuged at the speed of 1500 rpm for 10 min. and supernatant was collected.

Part II: Activation of Cadmium granules

1. Cadmium granules were stored in 0.1 mol/L H_2SO_4 solution.
2. At the time of assay the acid from granules was rinsed three times with deionized water.
3. Then the granules were swirled in 5 mmol/L $CuSO_4$ solution for 1-2 minutes.
4. These copper coated granules were drained and washed by glycine - NaOH buffer.
5. These activated granules were used within 10 minutes after activation.

6. The granules after use were washed by deionized water and stored in 0.1 mol/L H_2SO_4 solution. Same procedure for activation was followed each time.

Part III: Nitrite assay a set of three test tubes was arranged as follows and respective additions were made as follows.

Reagent	**Test**	**standerd**	**Blank**
Glycine-NaOH Buffer	500 µl.	500 µl.	500 µl.
Supernatant	500 µl.	--	--
Standard 10 µmol/L	--	500 µl.	--
D/W	--	--	500 µl.
Cadmium granules	**2**	**2**	**2**
Cadmium granules were swirled and tubes were kept at RT for 90 min.			
D/W	1.0 ml.	1.0 ml.	1.0 ml.
Content from all this tube was mixed well and diluted solutions were taken in following tubes			
Above diluted solutions	1.0 ml.	1.0 ml.	1.0 ml.
Sulfanilamide	0.5 ml.	0.5 ml.	0.5 ml.
N-napthylethylendiamine	0.5 ml.	0.5 ml.	0.5 ml.
After 20 minutes waiting all tubes were read at 540 nm.			

Calculation: Serum Nitrite (µm/L) = (T-B) ÷ (S-B) X 100

SPECIAL

HISTOLOGY

Fixation of tissues:

The fixation of tissues in 10% of formalin

Dissection and Fixation of Tissues:

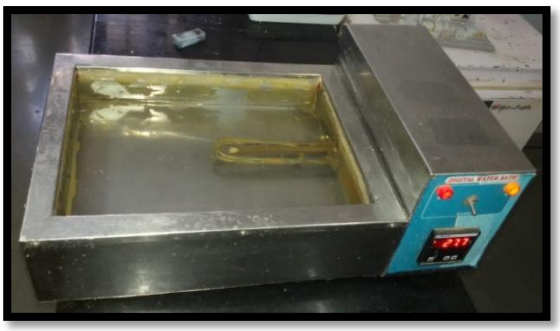
Rats were carefully dissected. Taking midline incision opened the anterior of the chest wall and neck. The thoracic cage opened and muscles were separated and the collected the blood directly from the heart and heart was removed. Then it was weighed immediately and fixed in 10% formalin. The heart was cut into pieces and fixed in bouins medium for 24 hrs. After fixation, it was placed in 70% alcohol for 6-8 hours during the day, then in 90% alcohol for overnight. Next day three changes of absolute alcohol were given for one hour each[19].

The tissue was blotted with blotting paper and placed in xylene for about 30 min for clearing. Then tissue was subjected to 3 changes of paraffin wax at 56^0 c temp 60^0 C for one hour each. Then tissue was embedded. 'L' shaped moulds were smeared with glycerine and fresh filtered wax poured into it to fill it almost. Any air bubbles formed were removed by hot spatula. Then the tissue was fixed on one side of the mould and label was placed on the opposite side of the mould. After a skin of wax has formed completely over the surface of the block, its solidification was hastened by careful immersion in cold water, for 15 min. Then the block was removed from the mould[19].

The blocks were prepared for cutting. Finally tissue was cut in the sizes of 5 to 7 μ in the form of a ribbon.

The individual sections were gently lowered onto the surface of water at 5^0 C to 10^0 C to remove the folds. The sections were taken on egg albumin coated slides. Slides were kept for drying on a hot plate at 45^0 c - 50^0 C for 2 hours or more as per requirement[19].

Microtome



Water bath

Hot Plate

Automatic tissue processing

Tissue collection:

Tissue collection for histopathology: after proper dissection of the animal heart (Atrium & Ventricle), aorta and muscular artery was isolated immediately and fixed in 10% neutral buffered formalin solution for 24 hours.[22] The fixed tissues were processed routinely and then embedded in paraffin, sectioned to 3–5 µm thickness, de-paraffinized, and rehydrated using standard techniques. The extent of hyperlipidemic (high fat diet) induced variations was evaluated by assessing morphological changes in the heart (Atrium & Ventricle), aorta and muscular artery sections stained with hematoxylin and eosin (H and E), using standard techniques[21].

Staining preparation:

1. Removal of paraffin wax was done by dipping into xylene one or two min in each of two changes of xylene.
2. Removal of xylene was done by dipping into two changes of absolute alcohol for one half to one min. each.
3. Then it was followed by treatment for a minute or two with 90% alcohol and then 70% alcohol.
4. After this, slides were kept under running tap water for about 5 minutes.
5. Then stained in haematoxylin for about 10-15 min and again kept under tap water for 5 min.
6. Excess stain was removed by dipping into acid alcohol for a few seconds. Here the blue colour was changed to red because of acid.
7. The blue colour (bluing) was regained by washing in alkaline, running tap water for 5-10 min. The stain was checked.
8. The slides were stained by eosin for 3-5min, surplus stain was washed off in water.
9. Dipping into ascending grades of alcohol did the dehydration of slides and clearing was done by two changes of xylene for about 1 min each.
10. The slides were mounted with DPX and cover slips were applied. The slides were kept at room temperature for some hours to allow, firm adhesion of the cover slip to the section.
11. The slides were observed under light microscope for microscopic differentiation and the photographs were taken
12. Few slides, were stained by verhoeffs stain technique.

VERHOEFF'S SPECIAL STAIN

This stain is useful in demonstrating atrophy of elastic tissue in cases of emphysema, and the thinning and loss of elastic fibres in arteriosclerosis, and other vascular diseases.

PRINCIPLE: The tissue is stained with a regressive hematoxylin, consisting of ferric chloride and iodine. The differentiating is accomplished by using excess mordant (ferric chloride) to break the tissue-mordant dye complex. The dye will be attracted to the larger amount of mordant in the differentiating solution and will be removed from the tissue. The

elastic tissue has the strongest affinity of the ironhematoxylin complex and will retain the dye longer than the other tissue elements.

CONTROL: skin.

FIXATIVE: Any well fixed tissue.

TECHNIQUE: Cut paraffin sections 4µ or 5µ.

EQUIPMENT: Rinse glassware in DI water: coplin jars, graduated cylinders

REAGENTS: Alcoholic Hematoxylin:

Hematoxylin 5.0 gm

Absolute alcohol 100.0 ml

Dissolve hematoxylin into alcohol with the aid of gentle heat, do not boil. Label with date and initials, solution are stable for 1 year.

CAUTION: Flammable, avoid contact and inhalation.

10% Ferric Chloride:

Ferric chloride 10.0 gm

Distilled water 100.0 ml mix well. Label with date and initials, solution are stable for 1 year.

CAUTION: Avoid contact and inhalation

Verhoeff's Hematoxylin:

Alcoholic hematoxylin 20.0 ml

10% ferric chloride 8.0 ml

Lugol's iodine 8.0 ml

Add in the order given, mixing between additions. Make fresh, discard.

Caution: Avoid contact and inhalation.

Differentiating Solution:

(2% Ferric Chloride) 10% ferric chloride 10.0 ml

Distilled water 40.0 ml make fresh, discard.

CAUTION: Avoid contact and inhalation 5% Hypo: See stock solutions

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.

2. Verhoeff's hematoxylin for 30 minutes (save solution until stain is completed)

3. Wash in tap water.

4. Differentiate in 2% ferric chloride solution, check microscopically for black fibers on a gray background.

5. Rinse in water.

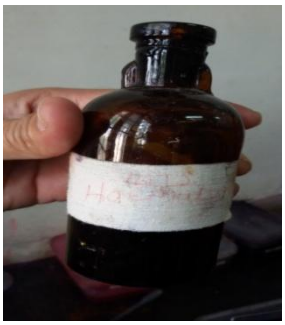
6. Hypo for 1 minute to remove iodine.

7. Wash in water.

8. Counterstain in van gieson's for 5 minutes.

9. Dehydrate, clear in xylene, and coverslip.

RESULTS: Elastic fibers and nuclei black collagen red other tissue elements yellow[22,23&24].



VASCULAR INTEGRITY PARAMETERS

Morphometric study

Elastic arterial wall thickness

- Tunica intima and media

Muscular arterial wall thickness

- Tunica intima and media

Coronary arterial wall thickness

Lumen diameter

- Anteroposterior
- Transverse
- Arterial lumen

Normalized wall index

Morphometry of Arterial Wall Thickness, Lumen Diameter and Calibration:

Procedure of Arterial Wall Thickness measurement in Histological:

Morphometry of arterial wall thickness & lumen diameter

- Arterial wall Thickness and its lumen diameter was measured by using Digimizer image analyzer version 4.3.0 copyright 2005-2014 Medclac software
- 8 megapixel Picture in 10X and 40X of microscopic (MIPS)and calibrated with Digimizer image and measured the thickness of arterial wall
- Whereas arterial lumen diameter was measured in 4X

Digimizer version 4.3.0 copyright C 2005-2014 Medclac softwere and following things were used

1. Strengthen the image.
2. Calibrate measurement
3. Performed the measured manually like: Length, Area, Centre, Unit, Area etc

4. Analyse the object

Average Intensity defined using the circle tool, area tool, rectangle tool digimizer can calculate the average intensity

Normalized wall index of coronary artery

- **Normalized Wall Index**
- The outer and inner vessel wall counters were manually traced for the coronary artery using the digimizer image analyzer software.
- Wall area, lumen area, and total vessel area were automatically calculated based on the counters drown by the software program.
- The normalized wall index was calculated by dividing the wall area by the total vessel area.
- $\frac{\text{Wall area}}{\text{Total Vessel Area}} =$ Normalized wall Index[25].

Before manual tracing the counter on arterial wall the 40X of microscopic image of the artery was calibarated with digimizer image analyzer.

DESCRIPTION OF CAMERA: ZEN 2 core package hardware license key with Axiocam 105 color (D) consisting of the camera Axiocam 105 color and the ZEN 2 core basic software plus the ZEN module image analysis camera specification: microscopy camera with driver software 64bit, USB 3.0 PCIe x1 interface, USB 3.0 connection cable 3.0 m, SATA power cable and molex power cable Sensor: Aptina CMOS color sensor basic resolution: 2560 (H) x 1920 (V) = 5.0 mega pixels color pixel size: 2.2 µm x 2.2 µm chip size: 5.70 mm x 4.28 mm, equivalent to 1/2.5" (diagonal 7.1 mm) Live frame rates (depending on hardware and software configuration): H x V binning factor frame rate@1ms 2560 x 1920 1 15 1280 x 960 2 37 640 x 480 4 47 frame rates for time series recording (depending on hardware and software configuration): H x V binning factor frame rate@1ms 2560 x 1920 1 8 1280 x 960 2 16 640 x 480 4 19 readout of sensor sub- regions ("ROI"):

Adjustable digitization: 3 x 8 Bit / Pixel integration Time: 100 µs up to 2 s interface (camera): USB 3.0 Micro-B Interface (PC / board): USB 3.0 standard a spectral range: Approx. 400 nm - 650 nm, IR filter read-out mode: Progressive optical interface: C-Mount (adapter 0.5x recommended) Size / Weight: approx. 2.9 cm x 2.9 cm x 4.76 cm / 50 g Housing: Aluminum, magnesium, stainless steel registration: CE, FCC class B, RoHS power supply: via USB 3.0 interface, max. 1.7 W (5 V, 0.34 A)[26].

MIPS (Magnified Image Processing System)

Environmental conditions: 0° ... +50° celsius, max. 80% relative air humidity, no condensation, free air circulation required Supported operating systems: Windows 7 x64 Prof./Ultimate SP1

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STATISTICAL ANALYSIS

STATISTICAL ANALYSIS:

- Values are expressed as Mean ± SD.
- To determine the significance between 5 groups, One Way ANOVA was applied.
- If one way ANOVA shows significant difference, then Post Hoc test was used to find the difference between two groups..
- Statistical significance was established at $P \leq 0.05$.
- Data were analysed by using SPSS software version 16.

CHAPTER 5

RESULTS

RESULTS:

Table 1. Photochemistry of *Terminalia Arjuna (Arjuna) &Emblica Officinalis (Amla)*

PHOTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *TERMINALIA ARJUNA (ARJUNA*) AND *EMBLICAL OFFICINLAIS(AMLA)*

CHEMICAL CONSTITUENTS PRESENT IN 99% ETHANOLIC EXTRACT OF *TERMINALIA ARJUNA* AND *EMBLICAL OFFICINLAIS*

S. NO	CHEMICAL CONSTITUENTS	99% ETHANOLIC EXTRACT *TERMINALIA ARJUNA*	99% ETHANOLIC EXTRACT *EMBLICAL OFFICINLAIS*
A	**Carbohydrates Test**		
1	Molisch's Test	**Negative (-ve)**	**Negative (-ve)**
2	Benedict Test	**Negative (-ve)**	**Negative (-ve)**
3	Fehling's Test	**Negative (-ve)**	**Negative (-ve)**
B	**Protein and Amino Acid Test**		
1	Xynthoproteic Test	**Negative (-ve)**	**Negative (-ve)**
2	Ninhydrin Test	**Negative (-ve)**	**Negative (-ve)**
3	Biuret Test	**Negative (-ve)**	**Negative (-ve)**
C	**Fixed Oil And Fats Test**		
1	Stain Test	**Negative (-ve)**	**Negative (-ve)**
2	Solubility Test	**Negative (-ve)**	**Negative (-ve)**
D	**Phytosterols Test**		
1	Solkowski's test	**Positive(+ve)**	**Positive(+ve)**
2	Libremenn Burchard's Test	**Positive(+ve)**	**Positive(+ve)**
E	**Glycosides Test**		
1	Modfied Borntrager's Test	**Positive(+ve)**	**Positive(+ve)**
2	Legal Test	**Positive(+ve)**	**Positive(+ve)**
F	**Flavonoids Test**		
1	Alkaline Reagent Test	**Positive(+ve)**	**Positive(+ve)**
2	Lead Acetate Test	**Positive(+ve)**	**Positive(+ve)**
3	Shinoda Test	**Positive(+ve)**	**Positive(+ve)**
4	Zinc Hydrochloric acid reduction test	**Positive(+ve)**	**Positive(+ve)**
G	**Alkaloids Test**		
1	Mayers Test	**Positive(+ve)**	**Positive(+ve)**
2	Wagner's Test	**Positive(+ve)**	**Positive(+ve)**
3	Dregandroff's Test	**Positive(+ve)**	**Positive(+ve)**
4	Hager's Test	**Positive(+ve)**	**Positive(+ve)**
H	**Tannins And Phenolic Compounds Test**		
1	Test With Dil. HNO3	**Positive(+ve)**	**Positive(+ve)**
2	Test With 5% of FeCl3	**Positive(+ve)**	**Positive(+ve)**
3	Lead Acetate Teast	**Positive(+ve)**	**Positive(+ve)**

Table 1. Phytochemical Analysis of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*):

After extractions the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery. (Positive) + = indicates present and (Negative) - = indicates absent

Different compounds of the *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* its phytochemical constituents are carbohydrates, tannins, phynoils, alkaloids, and saponins are identified.

GRAVIMETRY OF RATS

Table 2. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Gravimetry

% of Body Weight gain	Group1	Group 2	Group 3	Group 4	Group5	ANOVA	
						f. Value	p. Value
1st day	14.2±4.7	18.5±4.1	21±1.0[a]	15±3.8[a,c]	19.8±1.7[a]	5.2	0.003
21st day	14.5±2	16.8±1,4	8.9±3[a,b]	8.9±3[a,b]	14.6±2.1[c,d]	12.2	0.000

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3 and d depicts a comparison with group 5. (*p ≤0. 05).

Table 2. Gravimetry: Showing 1st day result there is a significant increase in % body weight gain in group 3, 4 and 5 compared to group 1(p≤ 0.05) . We observed significantly decrease in % body weight gain in group 4 compared to group 3(p≤ 0.05).

At 21st day there is a significant decrease in % body weight gain in group 3 and 4 compared to group 1 and 2 (p ≤0. 05). We observed a significant increase in % body weight gain in group 5 compared to group 3 and 4.

GENERAL

Table 3. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Haematology

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						f. value	p. value
Hb%	12.8±1.1	12.9±1.2	12.8±0.5	13.3±0.14	12±0.8	1.682	0.18
WBC	7783±2806	7266±2084	5633±1723	7383±2918	6566±1538	0.82	0.52
RBC	7.4±0.9	7.3±0.8	7.3±0.3	8±0.2	6.8±0.3[d]	2.667	0.03
Platelet	8.1±2.6	6.4±2.2	7.6±1.2	9.7±0.3	8.2±2.7	2.041	0.11
PCV	44.6±5.4	43.6±4.8	42.1±2.3	47.9±1.9	39.8±2.5[d]	3.895	0.01
MCHC	28.9±1.3	29.6±0.6	30.5±0.8	28.1±1.1[c]	30.3±1.7[d]	4.286	0.009

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5.(*p ≤0.05).

Table 3. Haematological parameters: there are no significant changes observed for Hb%, WBC and platelet among all groups. We have observed significant decrease in RBC, PCV and MCHC in group 5 when compared with group 4.

Biochemical Investigations:

LIPID PROFILE ANALYSIS

Table 4. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Lipid Profile

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F value	P value
TG mg/dl	140±35.9	200±93.2	110±23.5[b]	129±32.2	108±14.2[b]	3.58	0.01
TCmg/dl	122.8±16.1	123±17.5	131±15.8	185±17[a,b,c]	127.6±12.6[d]	16.5	0.000
HDLmg/dl	30±1.89	41.8±14[a]	29.6±1.8[b]	36.8±2.5	31±1.2	3.9	0.013
LDLmg/dl	64.7±18.5	39.0±7.12[a]	79.8±12.8[b]	122±14.13[a,b,c]	77±8.1[b,d]	33.3	0.000
VLDLmg/dl	28±7.18	40±18.6	22±4.7[b]	25.8±6.4	23±3.6[b]	3.2	0.02

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3 and d depicts a comparison with group 5. (*p ≤0. 05).

Table 4: We observed significantly lesser values of TG and VLDL in group 3 and 5 compared to group 2(*p ≤0.05). There was a significant increase in the values of TC and LDL compared to group 1, 2 and 3. TC levels have shown significant decreased values in group 5 compared to group 4. HDL levels have shown higher values in group 2 compared to group1. Also, we observed lesser levels of HDL in group 3 compared group 2(*p ≤0.05).

LIVER FUNCTION TEST:

Table 5. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Liver Function Test

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						f. value	p. value
Serum bilirubin mg%	0.61±0.1	0.78±0.1	0.8±0.1	0.68±0.19	0.8±0.1	1.96	0.13
Direct mg%	0.35±0.08	0.5±0.1	0.46±0.1	0.45±0.16	0.4±0.1	1.44	0.24
Indirectmg%	0.28±0.07	0.28±0.04	0.3±0.08	0.25±0.05	0.3±0.08	1.65	0.19
SGOT U/L	73±6.5	70±28	51.3±8.16	$18.6\pm5^{a,b,c}$	51 ± 8.1^{d}	13.7	0.000
SGPTU/L	60±19.7	64.5±12	47±5.67	$22.8\pm4^{a,b,c}$	47 ± 5.6^{d}	12.36	0.000
Serum protein gm/dl	5.7±0.2	5.7±0.2	5.6±0.24	6±0.3	5.6±0.2	1.67	0.18
Serum albumin gm/dl	2.9±0.1	3±0.19	2.8±0.13	$3.4\pm0.3^{a,b,c}$	2.8 ± 0.1^{d}	7.08	0.001
A/G ratio gm/dl	1±0.1	1±0.1	0.98±0.04	$1.3\pm0.2^{a,c}$	0.9 ± 0.04^{d}	5.79	0.002
Alkaline phos U/L	153±16.2	187±50	161±18.2	174±26	161±18	1.30	0.29

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5.(*p ≤0.05).

Table 5: We observed non significant differences for serum bilirubin, direct and indirect serum bilirubin, serum protein and alkaline phosphate between all groups. Serum SGPT and SGOT has shown significant decrease in levels in group 4 compared to group 1, 2 and 3(p≤ 0.05). There is a significant increase in SGPT and SGOT in group 5 compared to group 4. Levels of serum albumin have shown significantly higher values in group 4 compared to group 1, 2 and 3 (p≤ 0.05). There is a significant decrease in level of serum albumin in group 5 compared to

group 4. There is significantly increased in A/G ratio in group 4 compared to group 1 and 3 ($p \leq 0.05$). We observed significantly lesser levels of A/G ratio in group 5 compared to group 4.

TABLE 6. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Serum Na+, K^+ & Ca^{++}

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	Anova	
						F value	P value
Na+	143±5.07	140±1.5	139±1.86	141±4.9	139±1.6	1.26	0.31
K+	9.9±0.51	4.6±0.15[a]	4.86±0.7[a]	4.7±0.6[a]	4.9±0.7[a]	53.6	0.000
Ca++	5.4±2.18	8.7±0.1[a]	8.6±0.12[a]	8.7±0.2[a]	8.6±0.12[a]	13.18	0.000

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5.(*p ≤0.05).

Table 6: Levels of Na^+ has shown non significant differences among all groups. There is significant decrease in K^+ levels in group 2, 3, 4 and 5 compared to group 1($p \leq 0.05$). There is significant increase in values of Ca^{++} in group 2, 3, 4 and 5 compared to group 1 ($p \leq 0.05$).

Table 7. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Glucose Regulations

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	Anova	
						F value	P value
RBS mg/dl	98.5±26.8	118.8±13.8	100±18.6	115.6±15.7	96.1±15.6	1.94	0.134

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5.(*p ≤0.05).

To find out whether there is any impact of high fat diet on metabolic changes, we have estimated RBS (Random Blood Sugar) levels. RBS level in all the groups were found within normal range (table 7).

SPECIAL

SPECIALIZED

1. **Histology:**
    a. Atrium
    b. Ventricle
    c. Elastic Artery
    d. Muscular Artery
    e. Coronary Artery

HISTOLOGY OF ATRIUM:

G1. H&E stain 10X

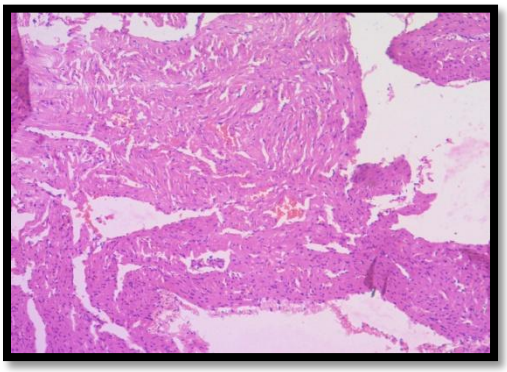


Figure 1: Atrium section of the control group revealing no pathological changes. HE **10x.**

G1. H&E stain 40X

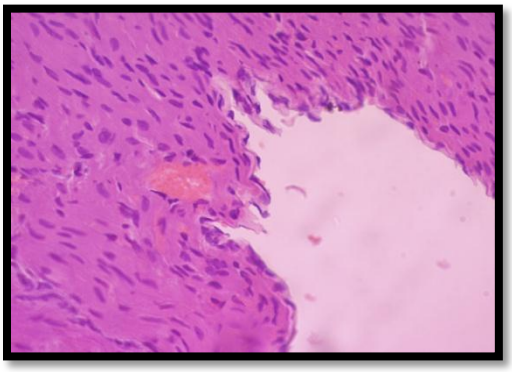


Figure2: Atrium section of the control group revealing no pathological changes. HE **40x.**

G2. H&E stain 10X s

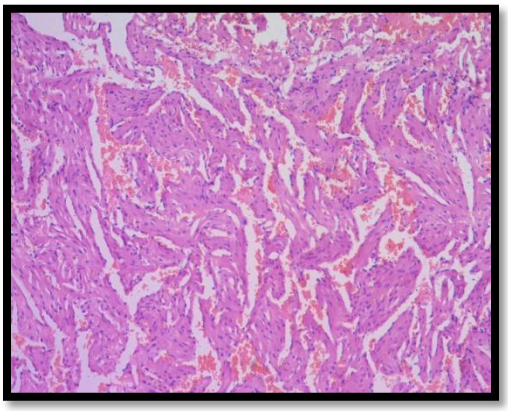


Figure3: Atrium section of Hyperlipidemic group revealing no pathological changes. HE **10x.**

G2. H&E stain 40X

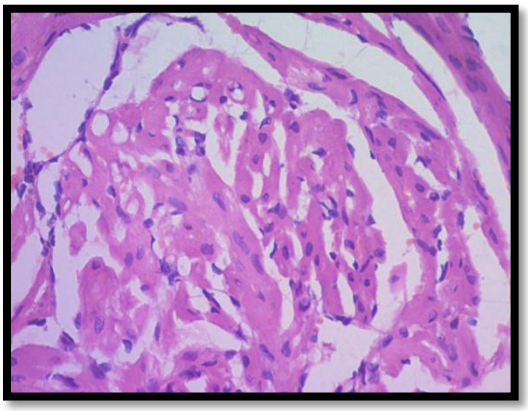


Figure4: Atrium section of Hyperlipidemic group revealing no pathological changes. HE **40x.**

G3. H&E stain 10X

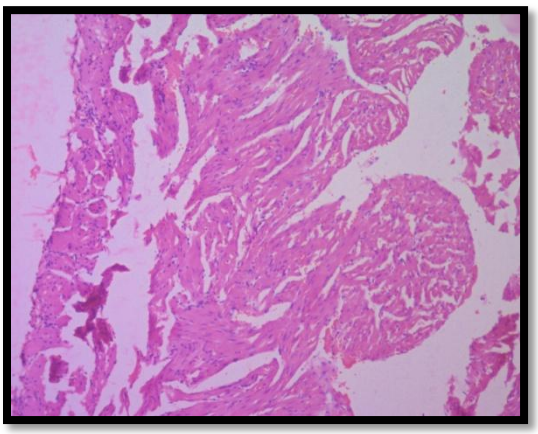


Figure5: Atrium section of Hyperlipidemic Treated with Terminalia Arjuna group revealing no pathological changes. HE **10x.**

G3. H&E stain 40X

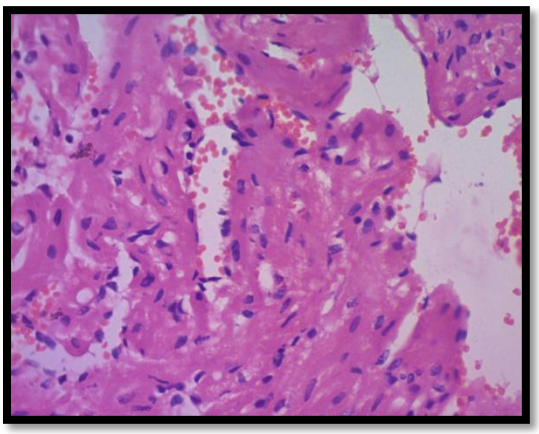


Figure6: Atrium section of Hyperlipidemic group Treated with Terminalia Arjuna revealing no Pathological changes. HE **40x.**

G4. H&E stain 10X

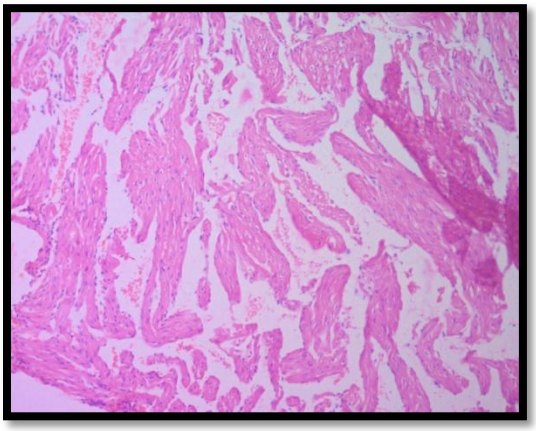


Figure7: Atrium section of Hyperlipidemic Treated with Emblica Officinalis group revealing no pathological changes. HE **10x.**

G4. H&E stain 40X

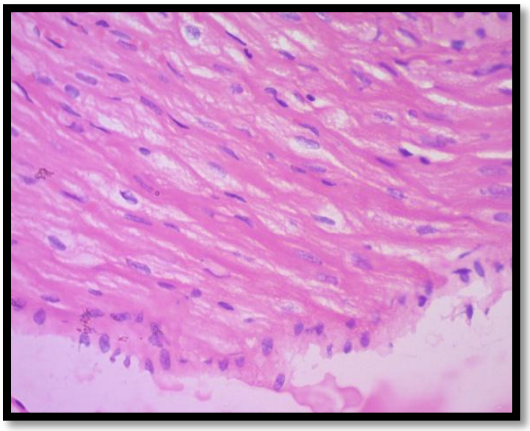


Figure8: Atrium section of Hyperlipidemic group Treated with Emblica Officinalis revealing no Pathological changes. HE **40x.**

G5. H&E stain 10X

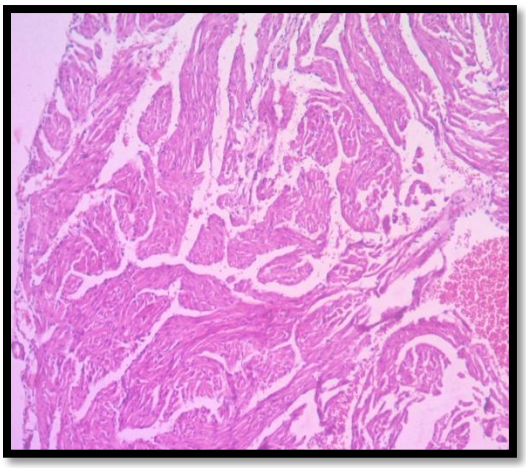


Figure9: Atrium section of Hyperlipidemic Treated with Terminalia Arjuna and Emblica Officinalis group revealing no pathological changes. HE **10x.**

G5. H&E stain 40X

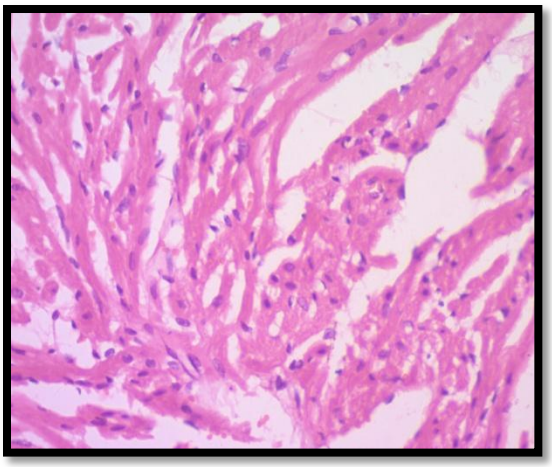


Figure10: Atrium section of Hyperlipidemic group Treated with Terminalia Arjuna and Emblica Officinalis revealing no Pathological changes. HE **40x.**

HISTOLOGY OF ATRIUM:

We have examined the histopathological study on atrium of different groups like Group 1 fed with Isocaloric diet, Group 2 fed with hyperlipidemic diet, Group 3 fed with hyperlipidemic diet and treated with Terminalia arjuna, Group 4 fed with hyperlipidemic diet and treated with Emblica Officinalis and the Group 5 rats fed with hyperlipidemic diet and treated both the drugs i.e. *Terminalia Arjuna (Arjuna) and Emblica Officinalis(Amla)*

The H&E stained section studied under the compound microscope and We observed that Atreal architecture of rats fed with Isocaloric diet i.e., group 1 showing healthy morphology of tissue.

The hyperlipidemic diet fed rats of group 2 showing normal architecture as such there is no damage, edema, leukocyte infiltration and necrosis was observed. The endocardial layer of atrium showing mild fat vacuoles is present in inner aspect of the wall.

We have even observed that the 3, 4 and 5 treated groups showing normal architecture or morphology of atrium hence there is no significant changes were observed in all groups of rats when compared to group 1.

HISTOLOGY OF VENTRICLE:

G1. H&E stain 10X

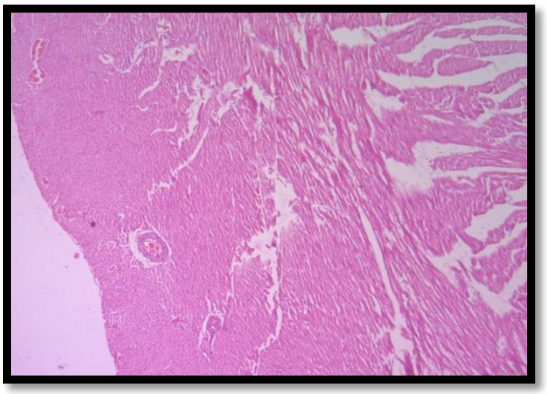


Figure 1: Ventricle section of the control group revealing no pathological changes. HE **10x.**

G1. H&E stain 40X

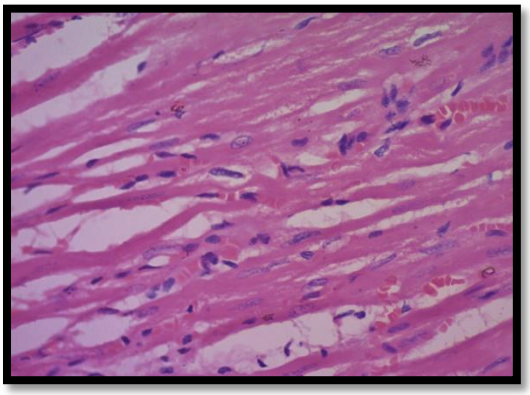


Figure 2: Ventricle section of the control group revealing no pathological changes. HE **40x.**

G2 H&E stain 10X

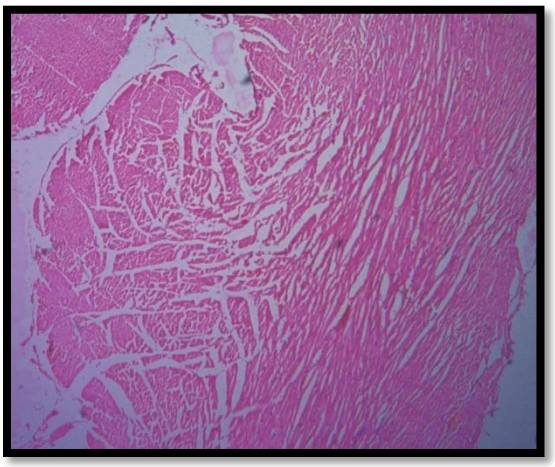


Figure 3: Ventricle section of Hyperlipidemic group revealing no pathological changes. HE **10x.**

G2 H&E stain 40X

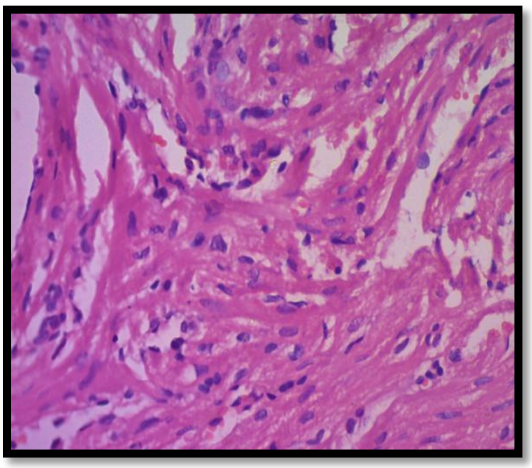


Figure 4: Ventricle section of Hyperlipidemic group revealing no pathological changes. HE **40x.**

G3 H&E stain 10X

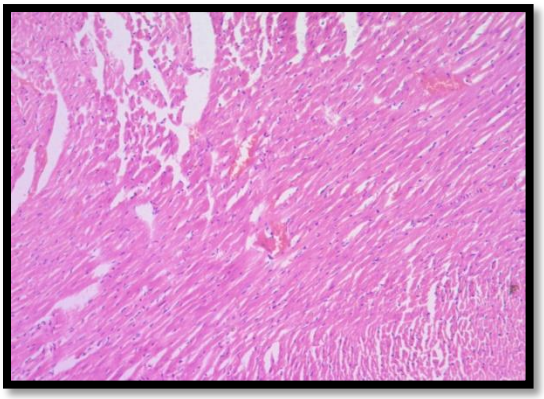


Figure 5: Ventricle section of Hyperlipidemic group Treated with *Terminalia Arjuna* revealing no pathological changes. HE **10x.**

G3 H&E stain 40X

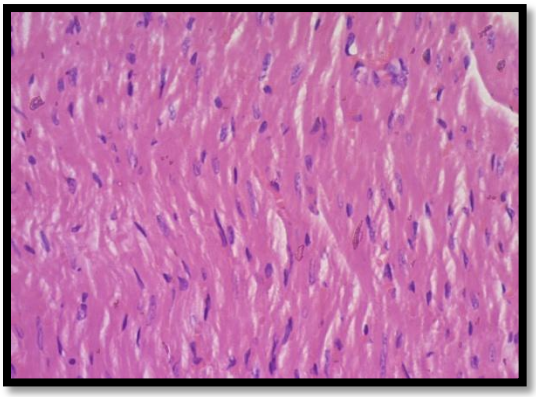


Figure 6: Ventricle section of Hyperlipidemic group Treated with *Terminalia Arjuna* revealing no pathological changes. HE **40x.**

G4 H&E stain 10X

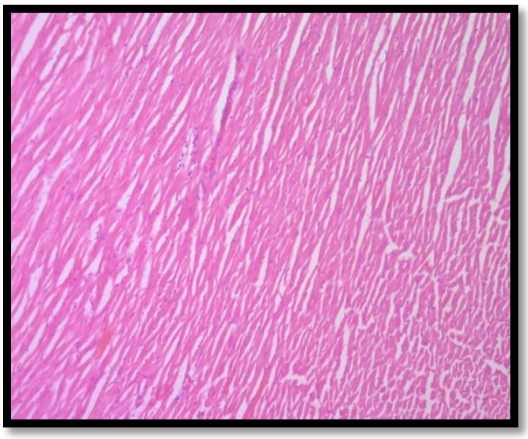


Figure 7: Ventricle section of Hyperlipidemic group Treated with *Emblica Officinalis* revealing no pathological changes. HE **10x.**

G4 H&E stain 40X

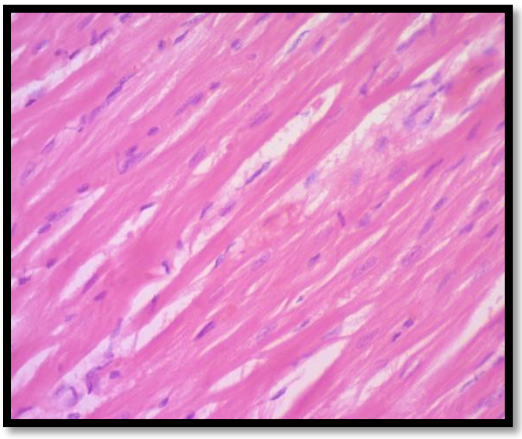


Figure 8: Ventricle section of Hyperlipidemic group Treated with *Emblica Officinalis* revealing no pathological changes. HE **40x.**

G5 H&E stain 10X

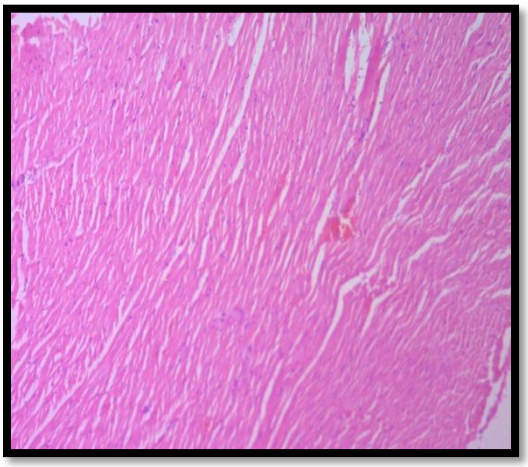


Figure 9: Ventricle section of Hyperlipidemic group Treated with *Terminalia Arjuna and Emblica Officinalis* revealing no pathological changes. HE **10x.**

G5 H&E stain 40X

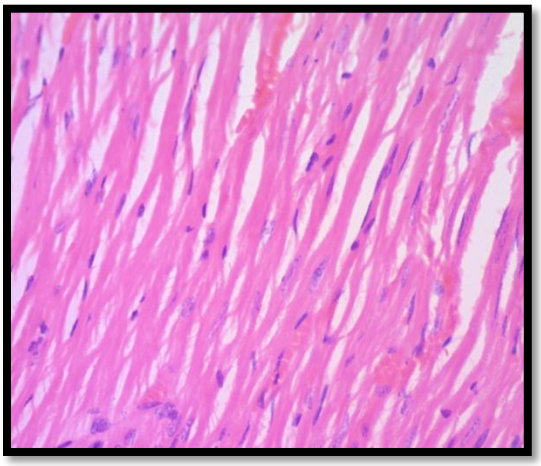


Figure 10: Ventricle section of Hyperlipidemic group Treated with *Terminalia Arjuna Emblica Officinalis* revealing no pathological changes. HE **40x.**

HISTOLOGY OF VENTRICLE:

The H & E stained section studied under the compound microscope and all the five groups of rats Ventricle we have examined the histopathological studies on myocardial tissue. The control group rats fed with Isocaloric diet showing the healthy morphological architecture of basal part of myocardial tissue within normal limits.

The group 2 hyperlipidemic diet fed rats showed no myocardial damage like edema, leukocyte infiltration and necrosis were observed. We observed in few slides, myocardium containing coronary artery showing an early change of atherosclerosis. However, these changes significantly decreased or not observed in treating groups.

We have observed that the group 3, 4 & 5. Are showing normal, healthy morphology of Ventricular musculature (Myocardium) when compared to group2

HISTOLOGY OF ELASTIC ARTERY:

G1 H&E STAIN 10X

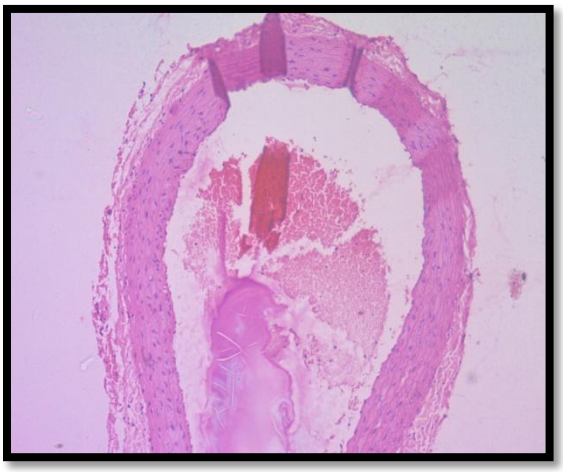


Figure 1: Elastic Artery section of the control group revealing no pathological changes. HE **10x**

G1 H&E STAIN 40X

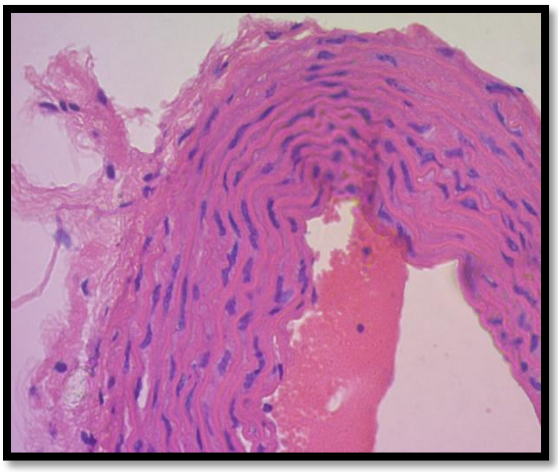


Figure 2: Elastic Artery section of the control group revealing no pathological changes. HE **10x**

G1 VEHEROFF'S STAIN 10X

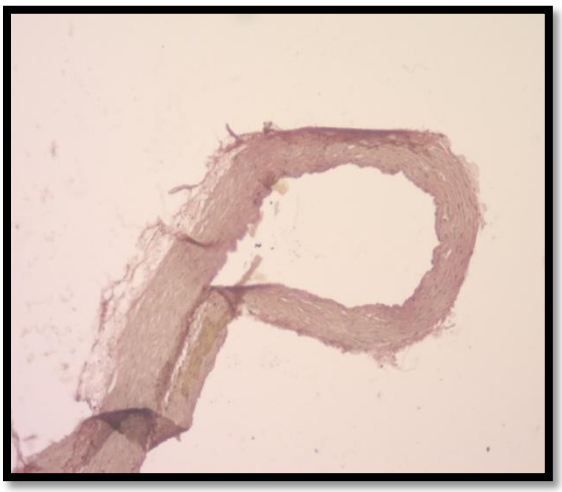


Figure 3: Elastic Artery section of the control group revealing no pathological changes. Veheroff's **10x**

G1 VEHEROFF'S STAIN40X

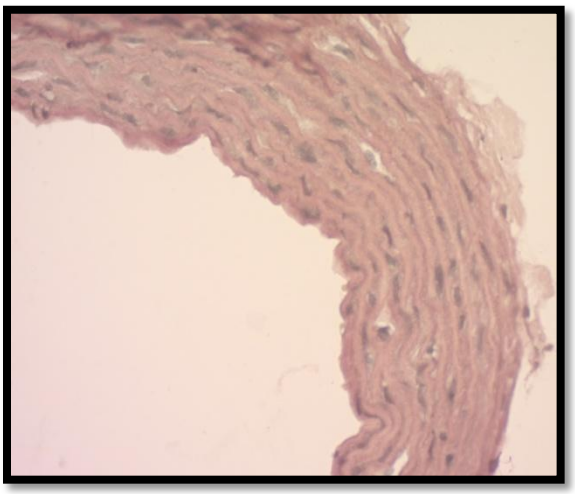


Figure 4: Elastic Artery section of the control group revealing no pathological changes. Veheroff's **40x**

G1 Elastic Artery 40x H&E stain

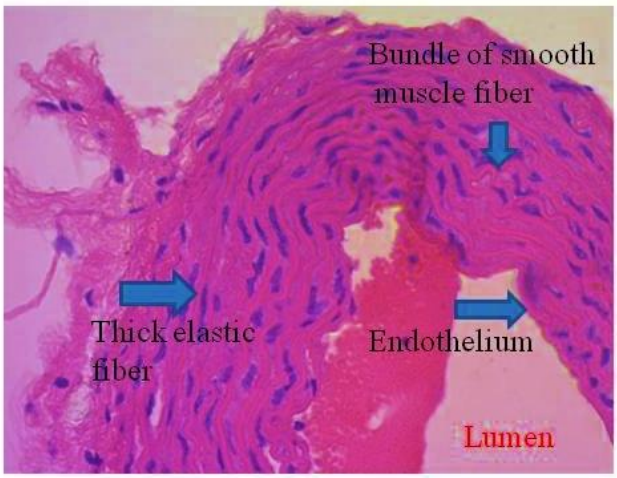


Figure 5: Elastic Artery section of Hyperlipidemic group revealing thin elastic fibre HE 10x

G2 Elastic Artery 40x H&E stain

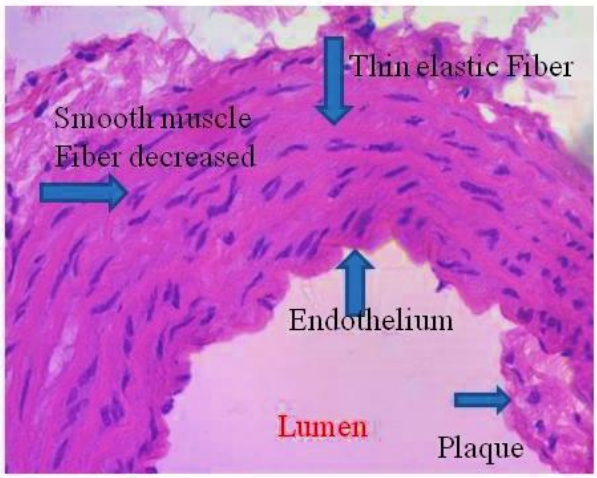


Figure 6: Elastic Artery section of Hyperlipidemic group revealing subintimal fat deposition HE 40x

G1 Elastic Artery 40x Veheroff's stain

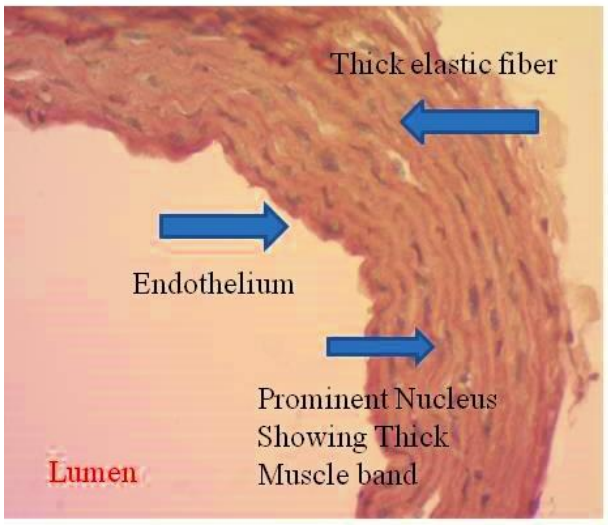


Figure 7: Elastic Artery section of Hyperlipidemic revealing reduced elastic fibres. Veheroff's 10x

G2 Elastic Artery 40x Veheroff's stain

Figure 8: Elastic Artery section of Hyperlipidemic group group revealing less reticular fibres and space increased space between reticular fibres Veheroff's 40x

G3 H&E stain 10X

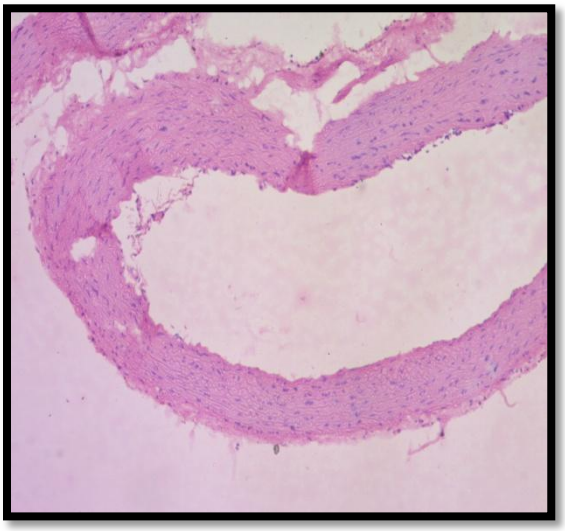


Figure 9: Elastic Artery section of Hyperlipidimic group treated with Terminalia Arjuna revealing no pathological changes. HE 10x

G3 H&E stain 40X

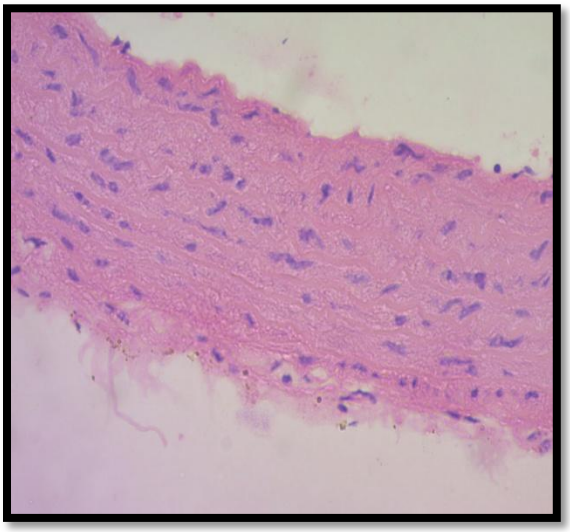


Figure10: Elastic Artery section of Hyperlipidimic group treated with Terminalia Arjuna revealing no pathological changes. HE 40x

G3 VEHEROFF'S STAIN 10X

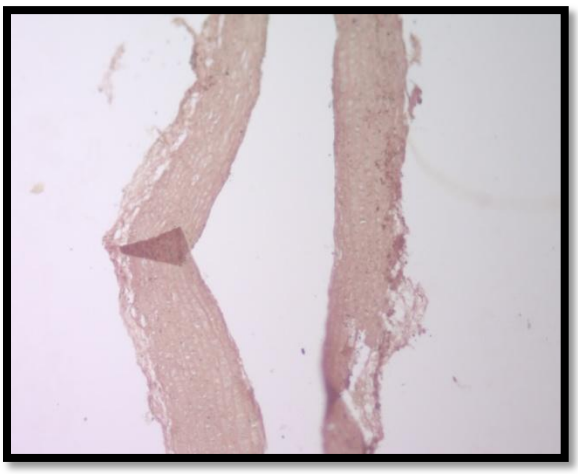


Figure11: Elastic Artery section of Hyperlipidemic group treated with Terminalia Arjuna revealing no pathological changes Veheroff's **10x**

G3 VEHEROFF'S STAIN40X

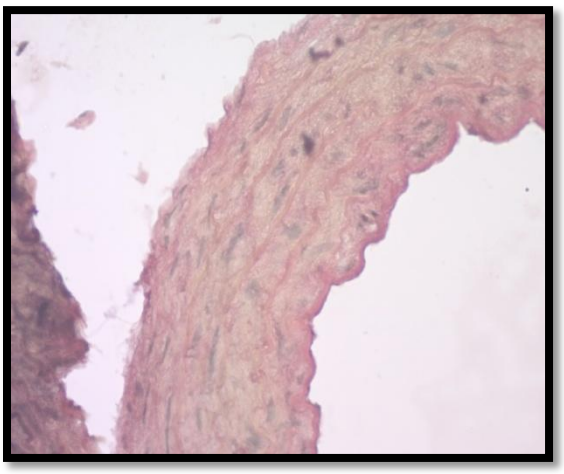


Figure12: Elastic Artery section of Hyperlipidemic group treated with Terminalia Arjuna. revealing no pathological changes Veheroff's **40x**

G4 H&E stain 10X

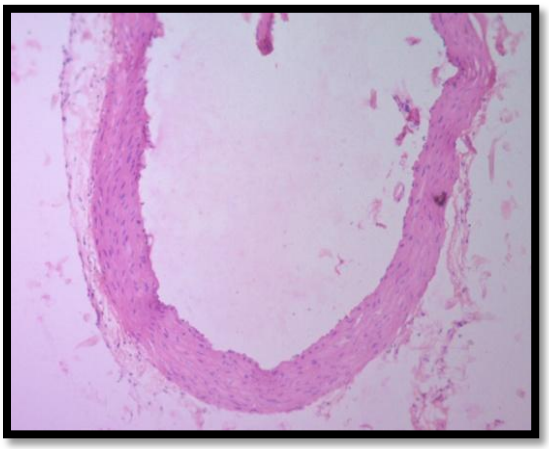


Figure13: Elastic Artery section of Hyperlipidimic group treated with *Emblica Officinalis* revealing no pathological changes. HE 10x

G4 H&E stain 40X

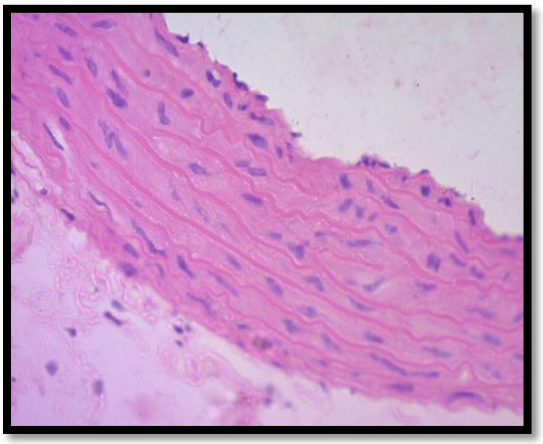


Figure14: Elastic Artery section of Hyperlipidimic group treated with *Emblica Officinalis* revealing no pathological changes. HE 40x

G4 VEHEROFF'S STAIN 10X

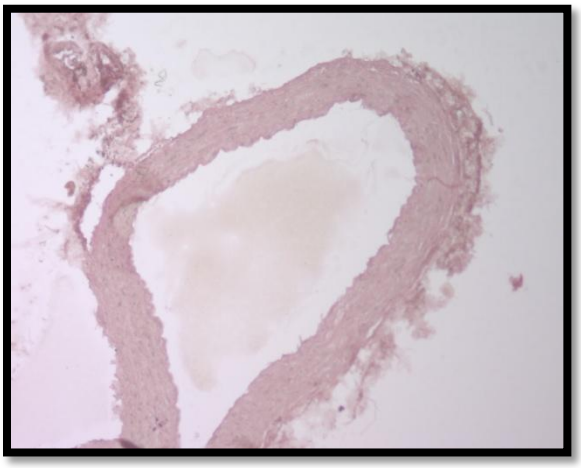


Figure15: Elastic Artery section of Hyperlipidemic group treated with *Emblica Officinalis* revealing no pathological changes Veheroff's 10x

G4 VEHEROFF'S STAIN40X

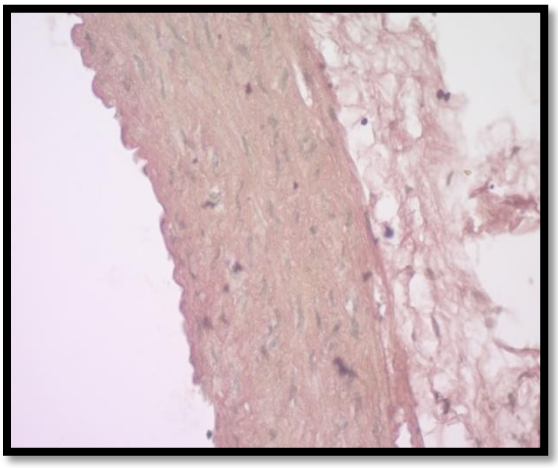


Figure16: Elastic Artery section of Hyperlipidemic group treated with *Emblica Officinalis*. revealing no pathological changes Veheroff's 40x

G5 H&E stain 10X

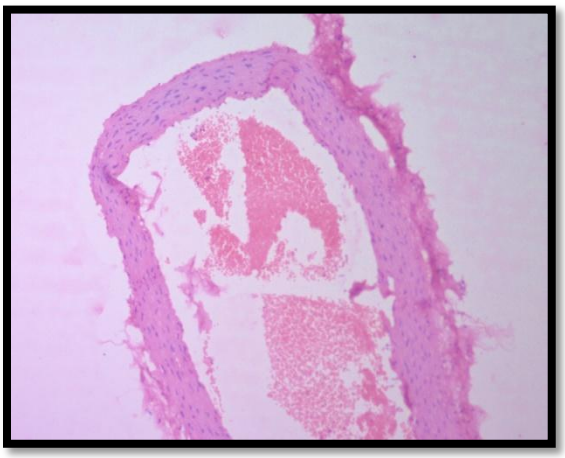


Figure17: Elastic Artery section of Hyperlipidimic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing no pathological changes. HE 10x

G5 H&E stain 40X

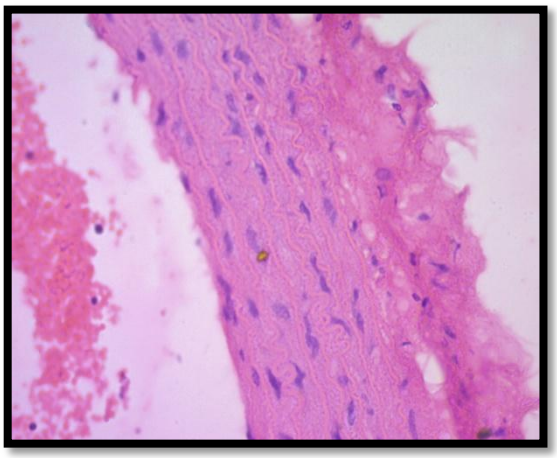


Figure18: Elastic Artery section of Hyperlipidimic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing no pathological changes. HE 40x

G5 VEHEROFF'S STAIN 10X

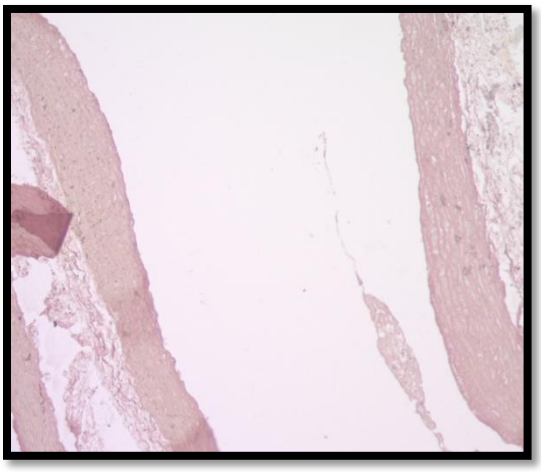


Figure19: Elastic Artery section of Hyperlipidemic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing no pathological changes Veheroff's 10x

G5 VEHEROFF'S STAIN40X

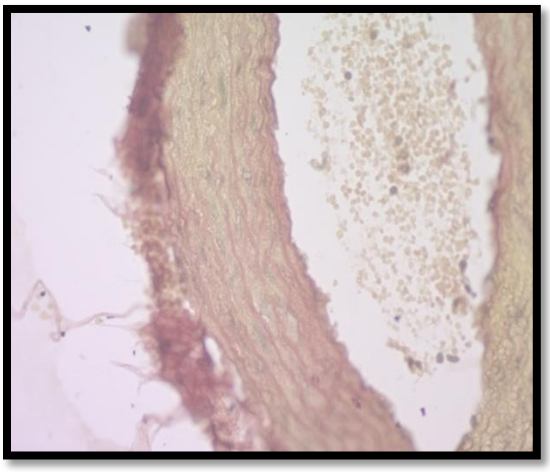


Figure20: Elastic Artery section of Hyperlipidemic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing no pathological changes Veheroff's 40x

HISTOLOGY OF ELASTIC ARTERY:

General microscopic structure of aorta in control and experimental groups

The H&E stained 10X and 40X section of the control/Isocaloric group showed typical 3 layers/tunicae of the aorta; the tunica intima with endothelial cells lining the tunica media with the normal arrangement of elastic lamellae and the horizontally oriented spindle-shaped nuclei of the smooth muscle cells and finally the tunica adventitia.

All the rats fed with a high fat diet group presented with morphological alterations of the smooth muscle cell nuclei in the tunica media. The tunica media showed the degeneration round shape and hyperplasia of the smooth muscle cell nuclei.

In addition to this, we observed subintimal atheromatus plaques were observed in some rats of (Fig 5 & 6 G2 H&E stain 10X and 40X) picture G2 H&E Stain 40X. In the hyperlipidemic fat fed rat group the histological structure of aorta was similar to that of the control group.

The H&E stained 10X and 40X microscopic structure of all 3 layers of the aorta (Tunica Intima, Media and Adventitia) of group 3 rats fed with high fat diet and treated with *Terminalia Arjuna (Arjuna)*, group 4 rats fed with high fat diet and treated with Emblica Officinalis and group 5 rats were fed with high fat diet and treated with *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)*, showing healthy architecture of elastic artery and significantly normal when compared with group 2.

Histological structure of rat aorta in control and experimental groups as revealed by Veheroff's stain

The Veheroff's stained sections showed the arrangement of elastic fibres of different studied groups, with relatively wide gaps between the elastic lamellae in the hyperlipidemic fat fed rat groups.

As compared with the control group (Fig 1& 2 G2 Veheroff's stain 10X & 40X) an increase of the volume of collagen fibres between two elastic lamellae in elastic artery wall was observed in group2 (Fig 3 & 4 Veheroff's stain 10X & 40X).

In addition to that there is decreased smooth muscle fibres and nucleus also in (Fig 3 & 4 Veheroff's stain 10X & 40X).

The Veheroff's stained sections shown the arrangement of elastic lamellae prominent and abundant in group 3, 4 & 5. Nuclei and smooth muscle fibres have observed normal and healthy. Hence group 3, 4 and 5 are significantly normal when compared to group 2

HISTOLOGY OF MUSCULAR ARTERY:

G1 H&E stain 10X

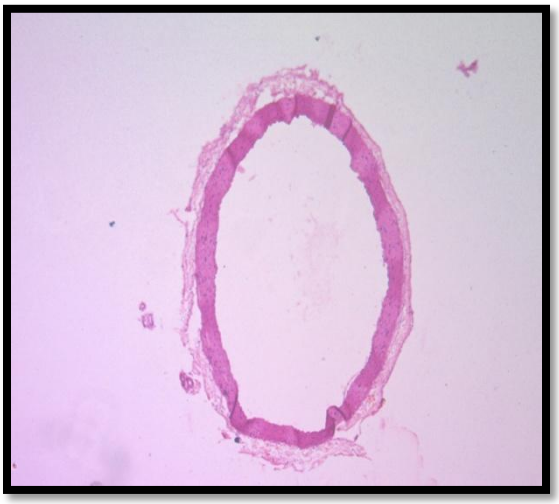


Figure 1: Muscular Artery section of the control group revealing no pathological changes. HE **10x**

G1 H&E stain 40X

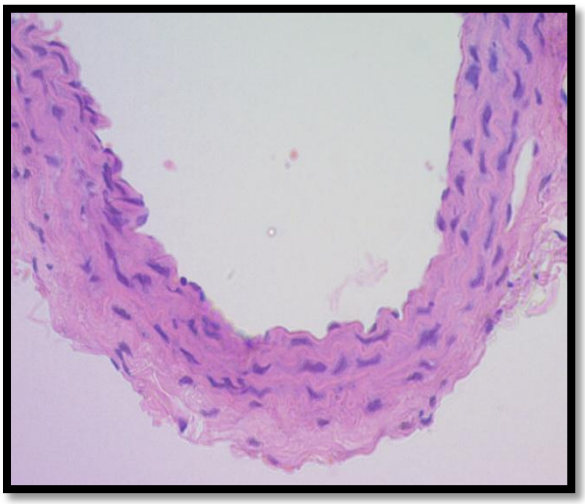


Figure 2: Muscular Artery section of the control group revealing no pathological changes. HE **10x**

G1 VEHEROFF'S STAIN 10X

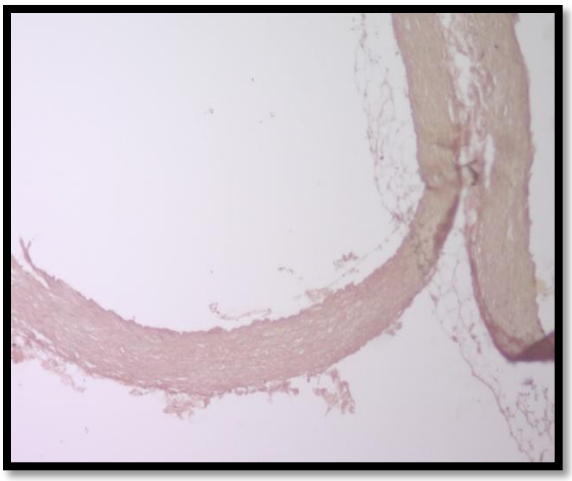


Figure 3: Elastic Artery section of the control group revealing no pathological changes. Veheroff's **10x**

G1 VEHEROFF'S STAIN40X

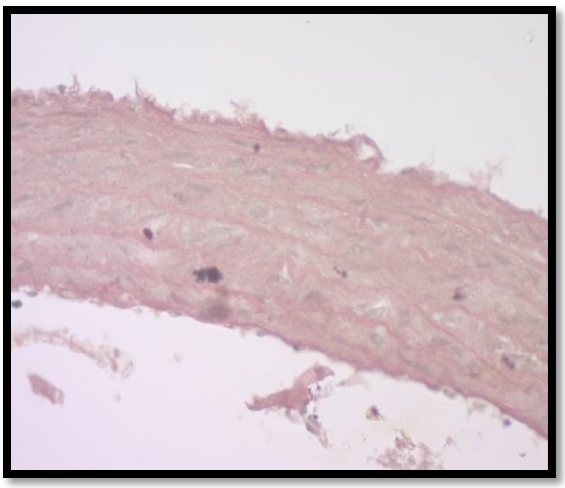


Figure 4: Elastic Artery section of the control group revealing no pathological changes. Veheroff's **40x**

G2 H&E stain 10X

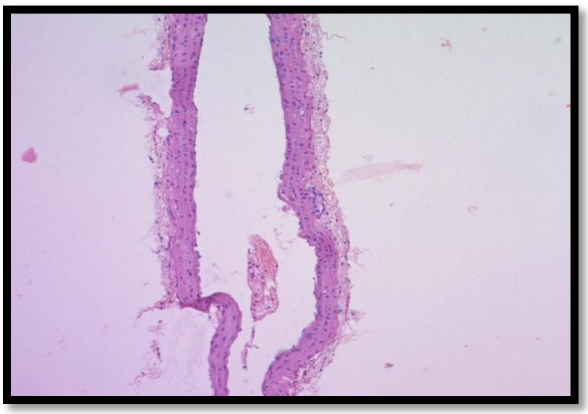


Figure 5: Muscular Artery section of Hyperlipidemic group revealing no pathological changes. HE **10x**

G2 H&E stain 40X

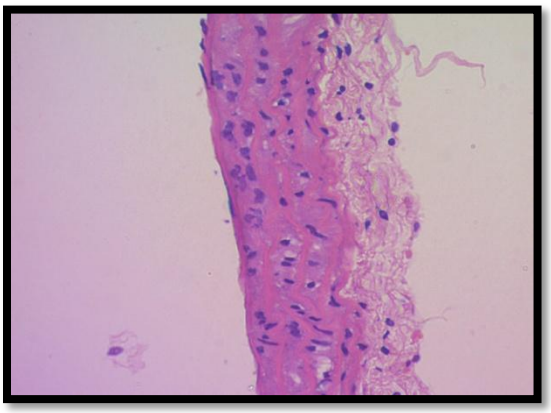


Figure 6: Muscular Artery section of Hyperlipidemic group revealing no pathological changes. HE **10x**

G2 VEHEROFF'S STAIN 10X

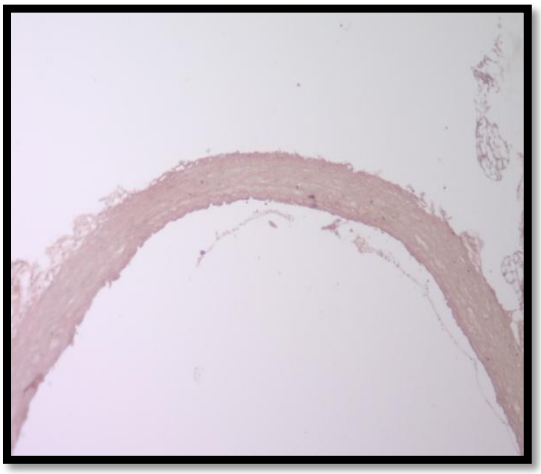


Figure 7: Muscular Artery section of Hyperlipidemic group revealing no pathological changes. Veheroff's **10x**

G2 VEHEROFF'S STAIN40X

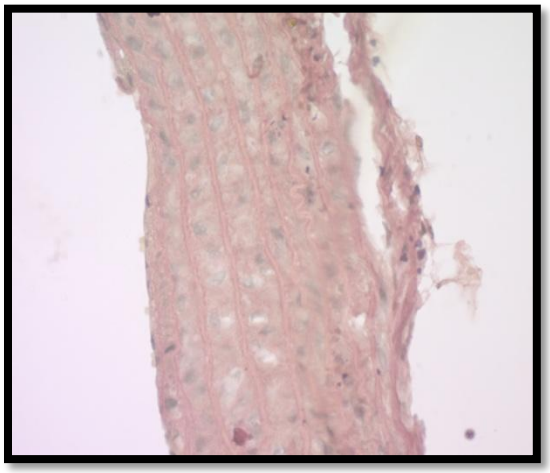


Figure 8: Muscular Artery section of Hyperlipidemic group revealing no pathological changes. Veheroff's **40x**

G3 H&E stain 10X

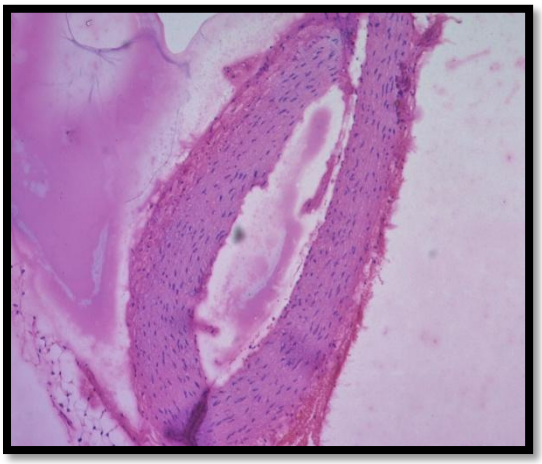


Figure 9: Muscular Artery section of Hyperlipidimic group treated with Terminalia Arjuna revealing no pathological changes. HE 10x

G3 H&E stain 40X

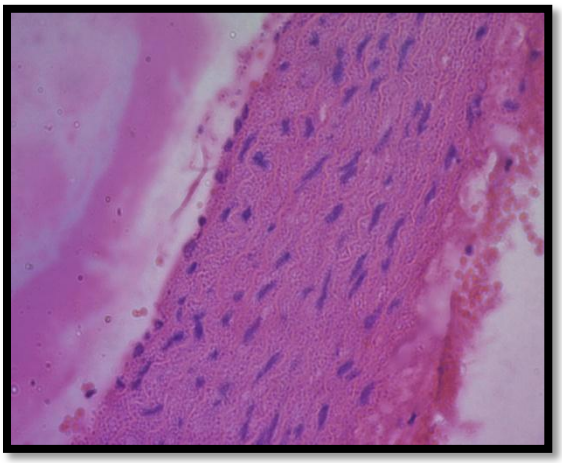


Figure10: Muscular Artery section of Hyperlipidimic group treated with Terminalia Arjuna revealing no pathological changes. HE 40x

G3 VEHEROFF'S STAIN 10X

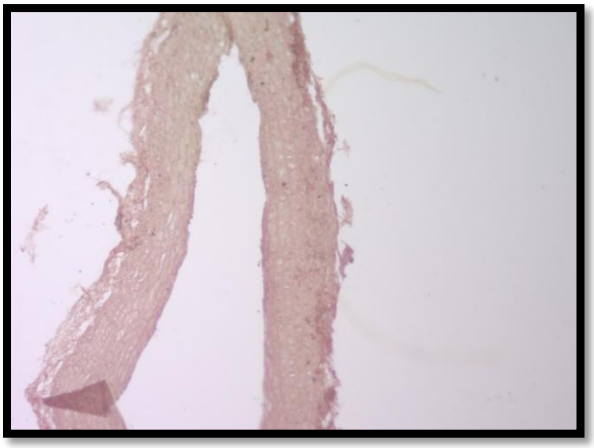


Figure11: Muscular Artery section of Hyperlipidemic group treated with Terminalia Arjuna revealing no pathological changes Veheroff's **10x**

G3 VEHEROFF'S STAIN40X

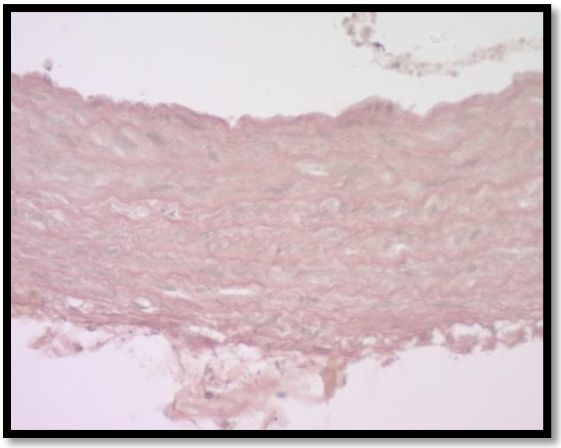


Figure12:Muscular Artery section of Hyperlipidemic group treated with Terminalia Arjuna. revealing no pathological changes Veheroff's **40x**

G4 H&E stain 10X

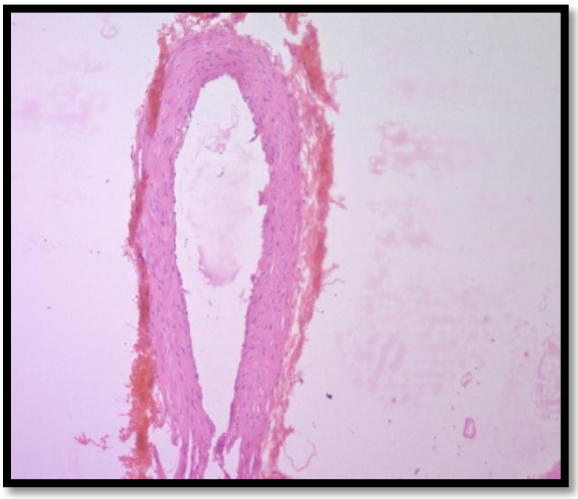


Figure13: Muscular Artery section of Hyperlipidimic group treated with *Emblica Officinalis* revealing no pathological changes. HE 10x

G4 H&E stain 40X

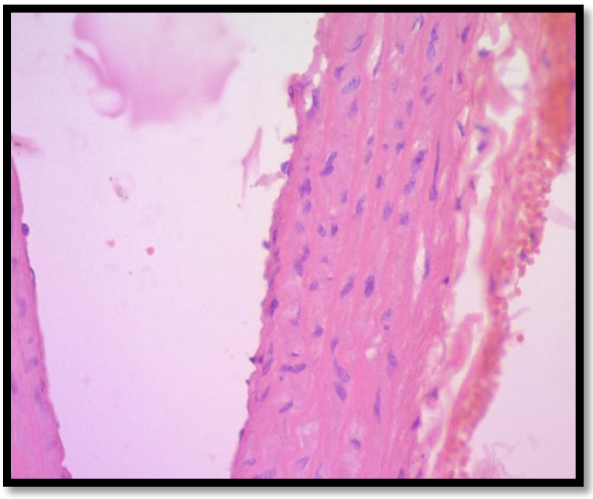


Figure14: Muscular Artery section of Hyperlipidimic group treated with *Emblica Officinalis* revealing no pathological changes. HE 40x

G4 VEHEROFF'S STAIN 10X

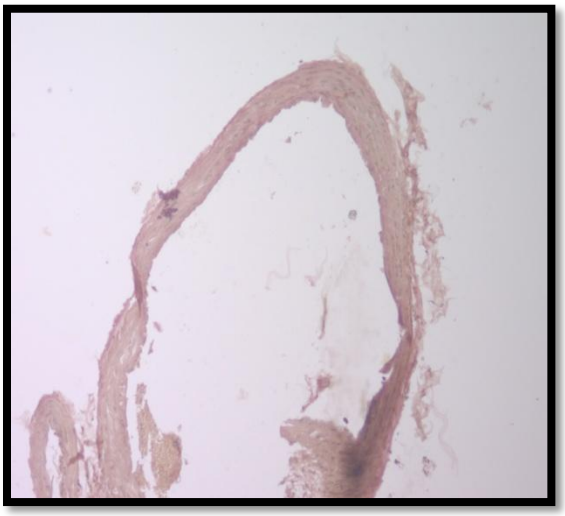


Figure15:Muscular Artery section of Hyperlipidemic group treated with *Emblica Officinalis* revealing no pathological changes Veheroff's 10x

G4 VEHEROFF'S STAIN40X

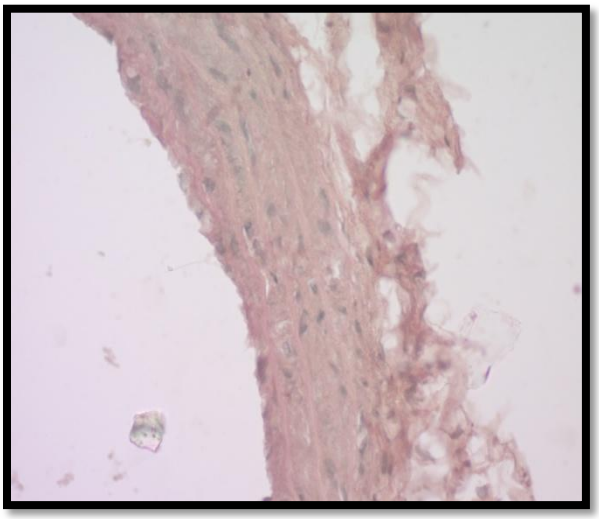


Figure16: Muscular Artery section of Hyperlipidemic group treated with *Emblica Officinalis*. revealing no pathological changes Veheroff's 40x

G5 H&E stain 10X

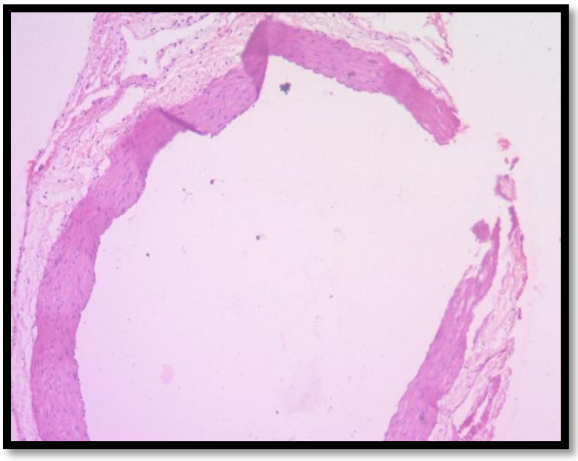


Figure17: Muscular Artery section of Hyperlipidimic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing no pathological changes. HE 10x

G5 H&E stain 40X

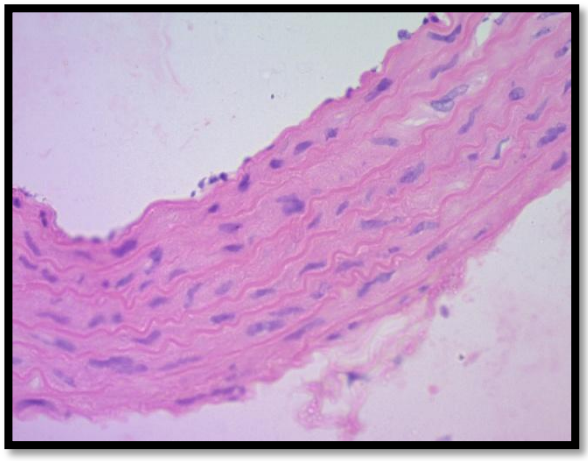


Figure18: Muscular Artery section of Hyperlipidimic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing no pathological changes. HE 40x

G5 VEHEROFF'S STAIN 10X

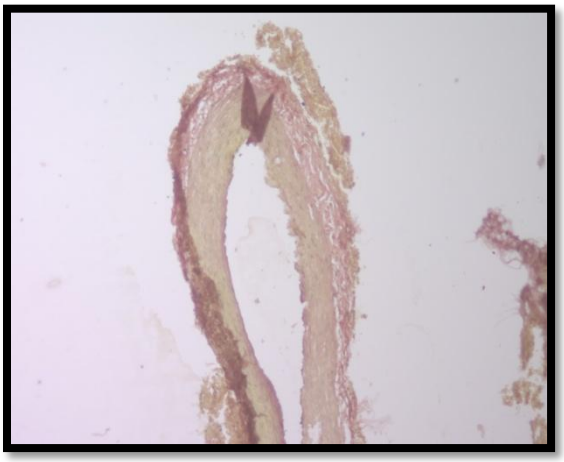


Figure19:Muscular Artery section of Hyperlipidemic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing no pathological changes Veheroff's 10x

G5 VEHEROFF'S STAIN40X

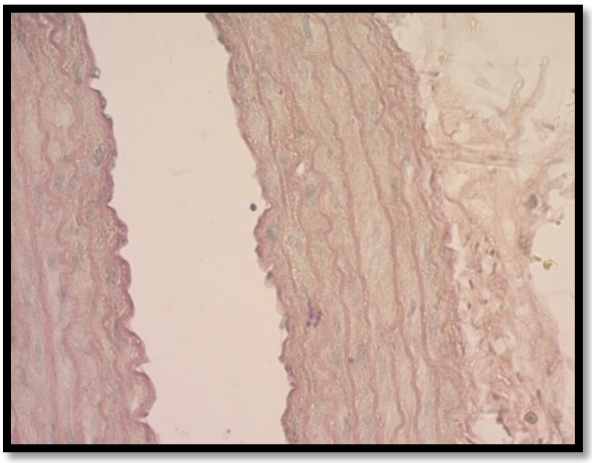


Figure20: Muscular Artery section of Hyperlipidemic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing no pathological changes Veheroff's 40x

HISTOLOGY OF MUSCULAR ARTERY:

General microscopic structure of Muscular artery in control and experimental groups

The H&E stained section of the control/Isocaloric group showed typical 3 layers of the Muscular artery; the tunica intima with endothelial cells lining the tunica media with the normal arrangement of elastic fibres and the horizontally oriented spindle-shaped nuclei of the smooth muscle cells and the outermost layer is tunica adventitia.

All the rats of group 2, fed with a high fat diet, presented the normal morphological architecture of the smooth muscle cell and nuclei in the tunica media.

We observed in the high fat fed rat group 2 the histological structure of Muscular artery was similar to that of the control group.

The H&E stained microscopic structure of all 3 layers of the Muscular artery (Tunica Intima, Media and Adventitia) of group 3 rats fed with high fat diet and treated with *Terminalia Arjuna (Arjuna),* group 4 rats fed with high fat diet and treated with *Emblica Officinalis(Amla)* and group 5 rats were fed with high fat diet and treated *Terminalia Arjuna(Arjuna) and Emblica Officinalis(Amla),* showing healthy architecture of Muscular artery and significantly normal when compared with group 1.

Histological structure of rat Muscular artery in control and experimental groups as revealed by Veheroff's stain

The Veheroff's stained sections showed the arrangement of elastic fibres of different studied groups, with relatively mild gaps between the elastic lamellae in the high fat fed rat groups.

As compared with the control group (Fig 3. G1 Veheroff's stain 10X and. Fig 4. G1 Veheroff's stain 40X) a slight increase in the volume of collagen fibres between two elastic lamellae in the Muscular artery wall was observed in group2.

In addition to that there is mild decreased smooth muscle fibres and nucleus also in (Fig 7. G2 Veheroff's stain 10X and. Fig 8. G2 Veheroff's stain 40X).

The Veheroff's stained sections of a muscular artery shown the arrangement of elastic lamellae normal in group 3, 4 & 5. Nuclei and smooth muscle fibres were abundantly observed normal and healthy. Hence group 3, 4 and 5 are significantly normal when compared to group 1. (Fig 11, 12, 15, 16 19 and 20. G3, G4 and G5 Veheroff's stain 10X and. Fig 8. G3, G4 and G5 Veheroff's stain 40X).

CORONARY ARTERIAL HISTOPATHOLOGY:

GROUP 1 H&E STAIN 10X

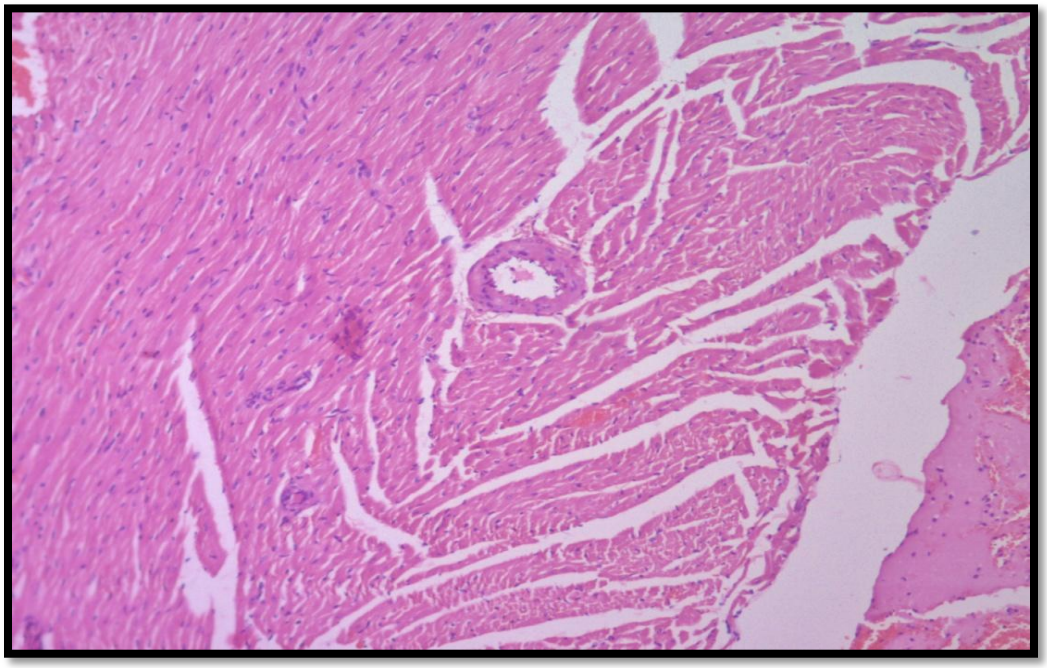


Figure 1: Ventricle section of the control group revealing Coronary Artery Normal histology H&E 10X

GROUP 1 H&E STAIN 40X

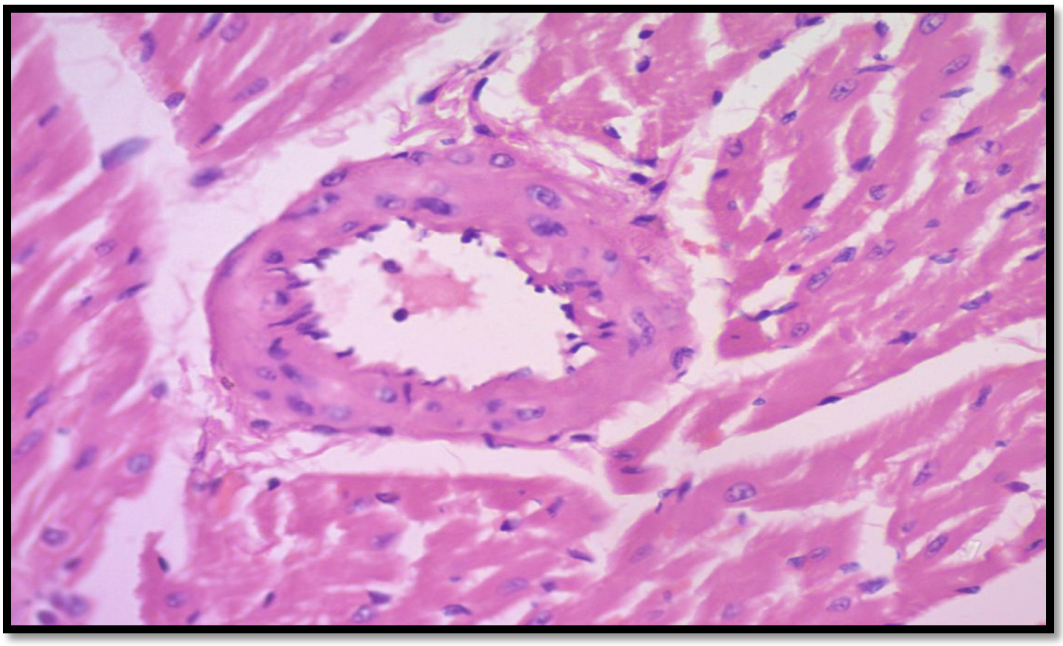


Figure 2: Ventricle section of the control group revealing Coronary Artery Normal histology H&E 40x

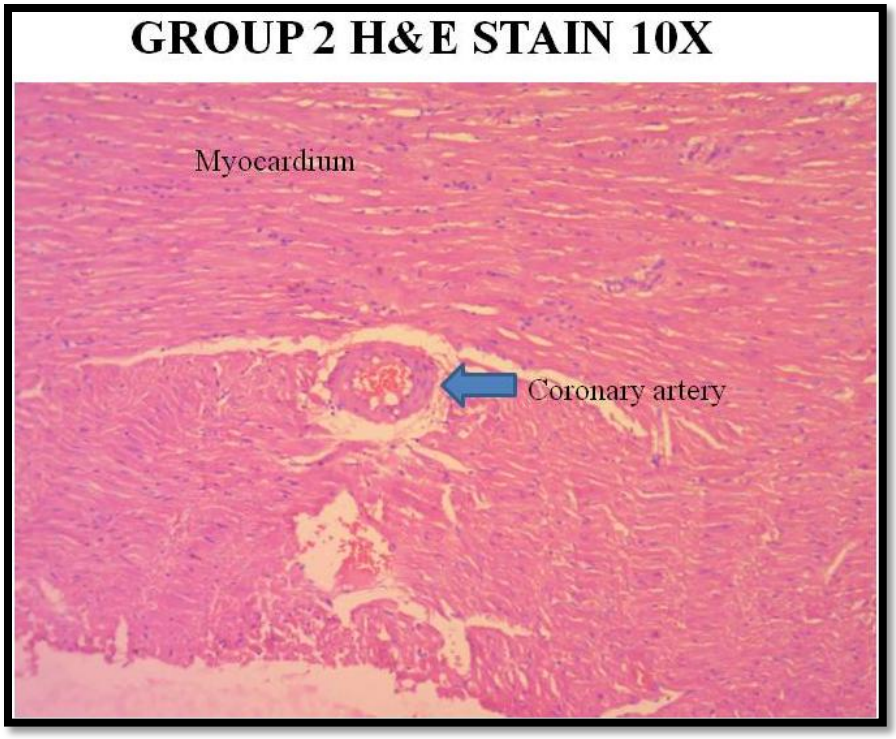


Figure 3: Ventricle section of the Hyperlipidimic group revealing thickened Coronary Artery. HE **10X**

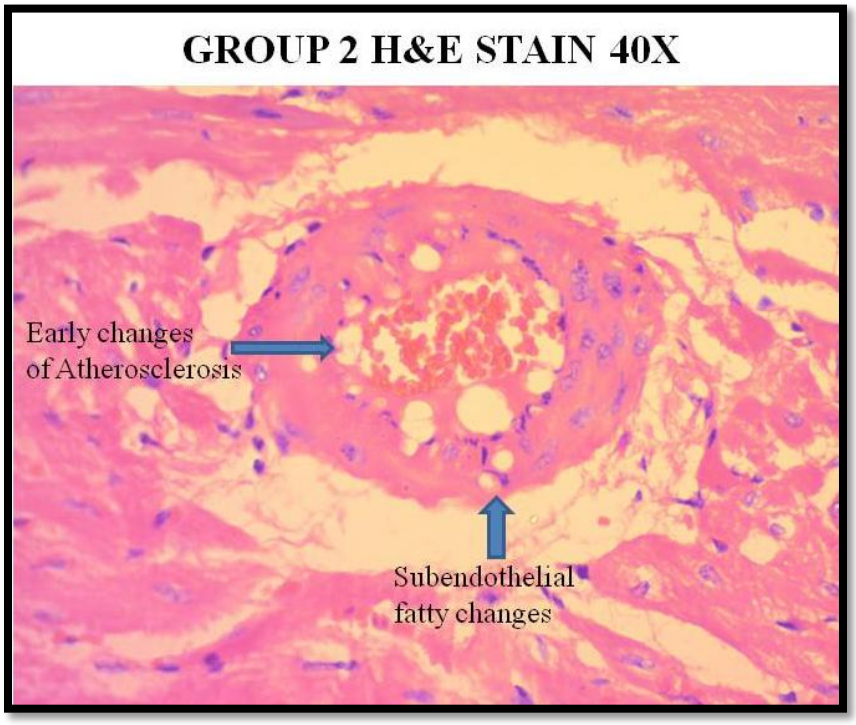


Figure 4: Ventricle section of the Hyperlipidimic group revealing subintimal deposition of fat in Tunica intima and Tunica Media. HE 40X

GROUP 3 H&E STAIN 10X

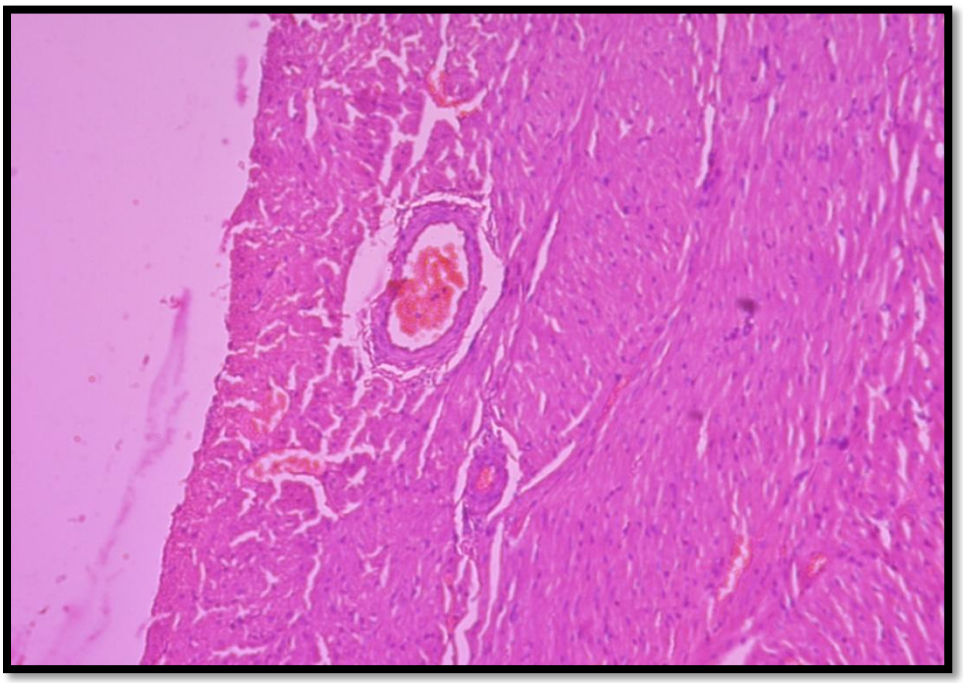


Figure 5: Ventricle section of the Hyperlipidimic group treated with *Terminalia Arjuna* revealing Coronary Artery pathological changes. HE **10x**

GROUP 3 H&E STAIN 40X

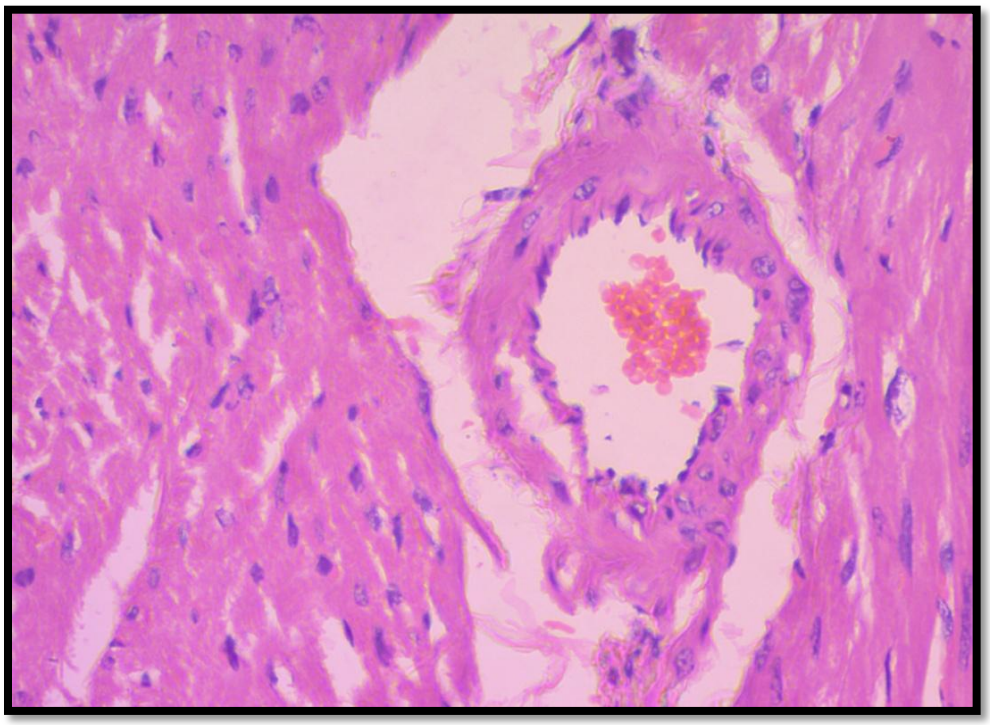


Figure 6: Ventricle section of the Hyperlipidimic group treated with *Terminalia Arjuna* revealing Coronary Artery pathological changes. HE 40x

GROUP 4 H&E STAIN 10X1

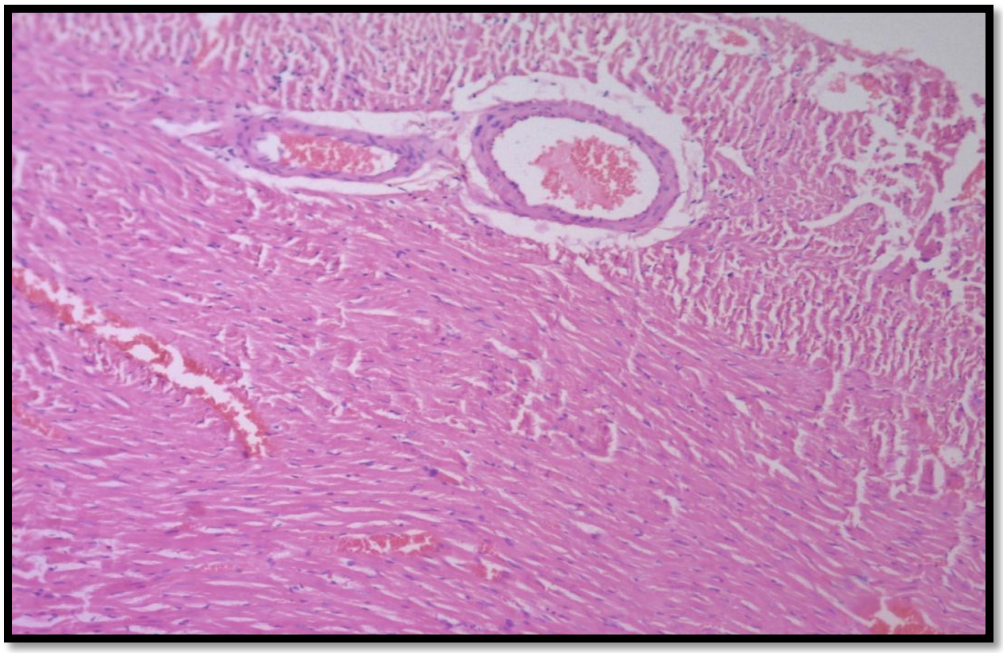


Figure 7: Ventricle section of the Hyperlipidimic group treated with *Emblica Officinalis* revealing Coronary Artery pathological changes. HE 10x

GROUP 4 H&E STAIN 40X

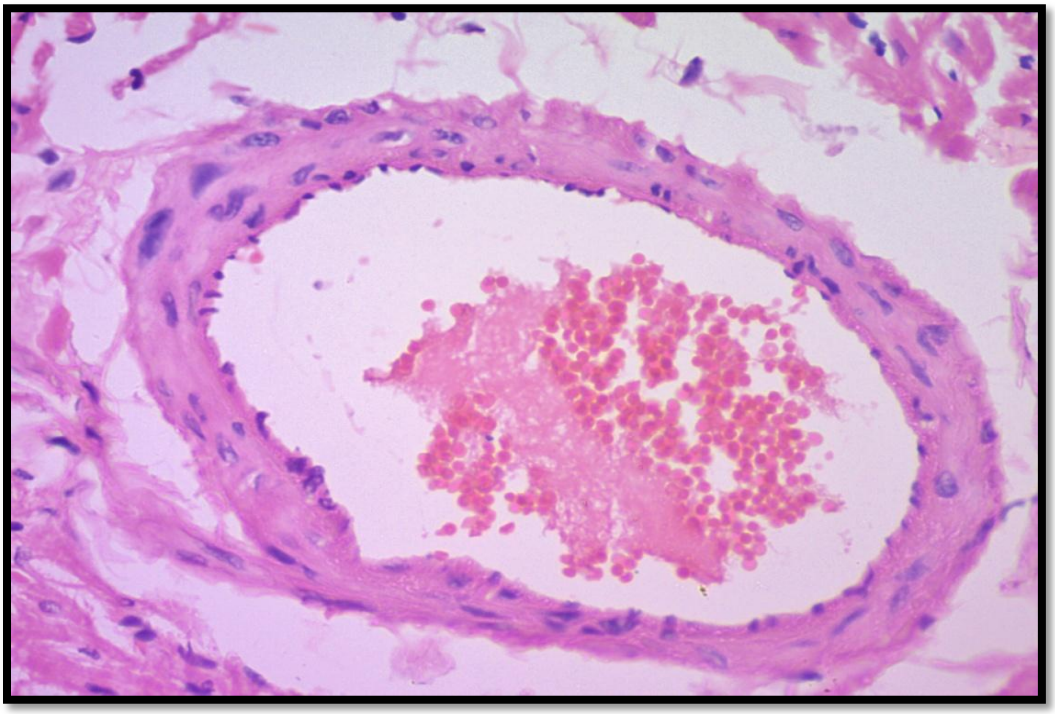


Figure 8: Ventricle section of the Hyperlipidimic group treated with *Emblica Officinalis* revealing Coronary Artery pathological changes. HE **40x**

GROUP 5 H&E STAIN 40X

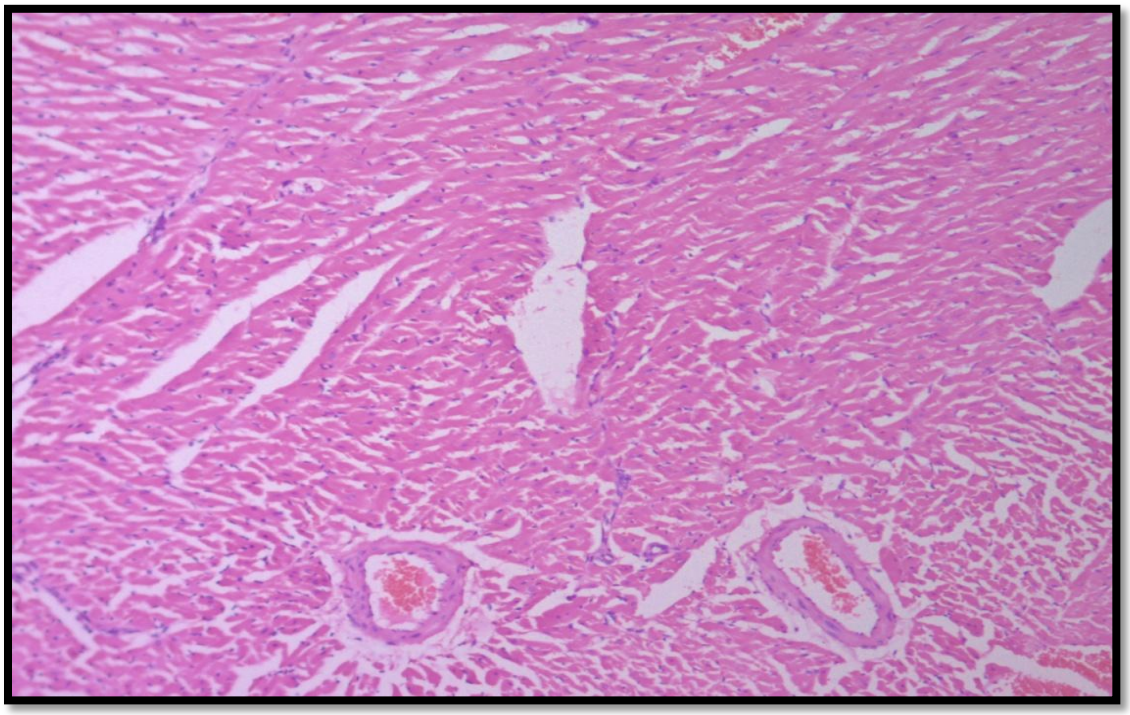


Figure 9: Ventricle section of the Hyperlipidimic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing Coronary Artery pathological changes. HE **10x**

GROUP 5 H&E STAIN 40X

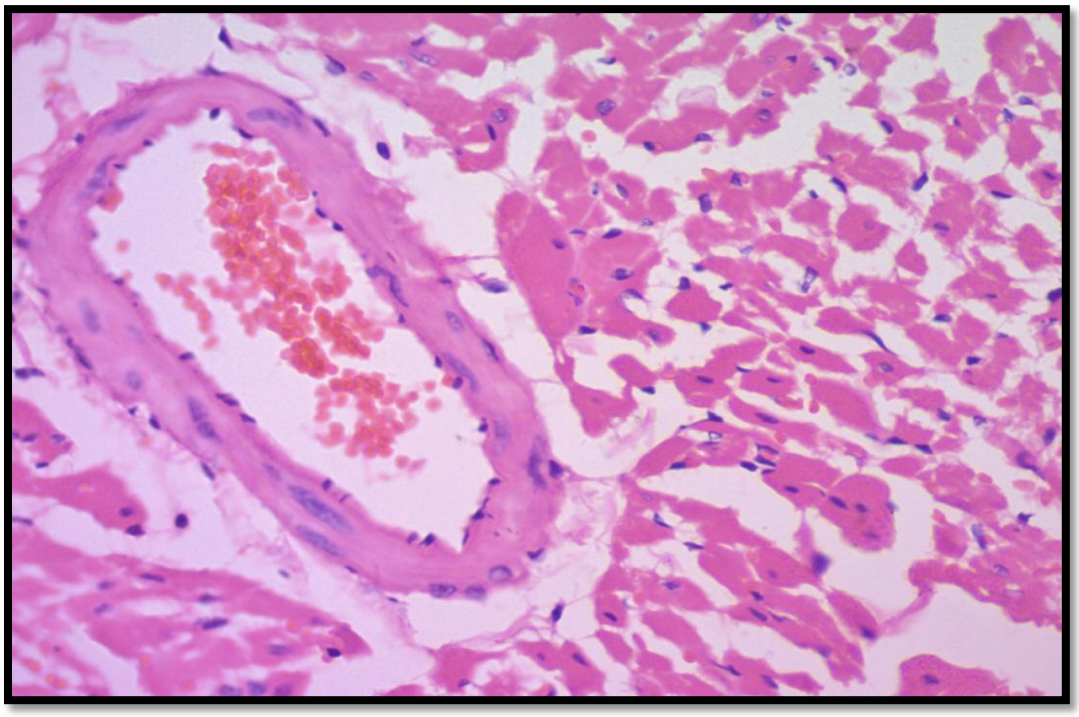


Figure 10: Ventricle section of the Hyperlipidimic group treated with *Terminalia Arjuna* and *Emblica Officinalis* revealing Coronary Artery pathological changes. HE 40x

CORONARY ARTERIAL HISTOPATHOLOGY:

The H & E stained section Myocardium containing coronary artery of control/Iso-caloric diet fed rats group 1, showing normal and healthy microscopic architecture. The tunica intima with the endothelial cells lining the tunica media with normal arrangement of internal elastic lamellae and the well defined spindle shaped smooth muscle cells in 10X and 40X. (Fig No 1 & 2 G1 H&E stain of CA)

(Fig No 3 & 4 G2 H&E stain of CA) Coronary artery of group 2 rats fed with high fat diet presented with morphological alterations in the lumen and arterial wall. We have observed that the morphologic appearance of coronary arterial lumen in group 2 rats fed with high fat diet showing an early change of atherosclerotic plaques. The atherosclerotic plaques are in the form of fatty changes in the Subendothelial layer as well as in the arterial wall. Hence coronary arterial wall showed mild degeneration, round shaped hyperplasia of smooth muscle cells nuclei.

We even observed that the coronary arterial wall thickness is significantly increased in group 2 compared to group 1. Whereas coronary artery wall thickness group 3, 4 and 5 (Fig No G3 5&6, G4 7&8 and G5 9&10 H&E stain of CA) significantly decreased and showing healthy normal architecture when compared to group 2.

Group 3 high fat diet fed rats treated with *Terminalia Arjuna (Arjuna)*, group 4 high fat diet fed rats with treated *Emblica Officinalis (Amla)* and group 5 high fat diet fed rats treated with *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* shows a remarkable improvement of architecture of coronary arterial wall as compared to group 2.

2. VASCULAR INTEGRITY BASED ON HISTOLOGICAL FROFILE:

a. **Estimation of Elastic artery Thickness:** Tunica Intima, Tunica Media.

Estimation of Elastic Artery lumen Diameter

i. Transverse ii. Anteroposterior and iii. lumen diameter

b. **Estimation of Muscular artery Thickness:** Tunica Intima, Tunica Media wall thickness.

c. **Estimation of Coronary Artery Thickness:** Wall thickness

Estimation of Coronary artery lumen Diameter

ii. Transverse ii. Anteroposterior and iii. lumen diameter

d. Normalized Wall Index

TABLE8. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Elastic Artery Thickness

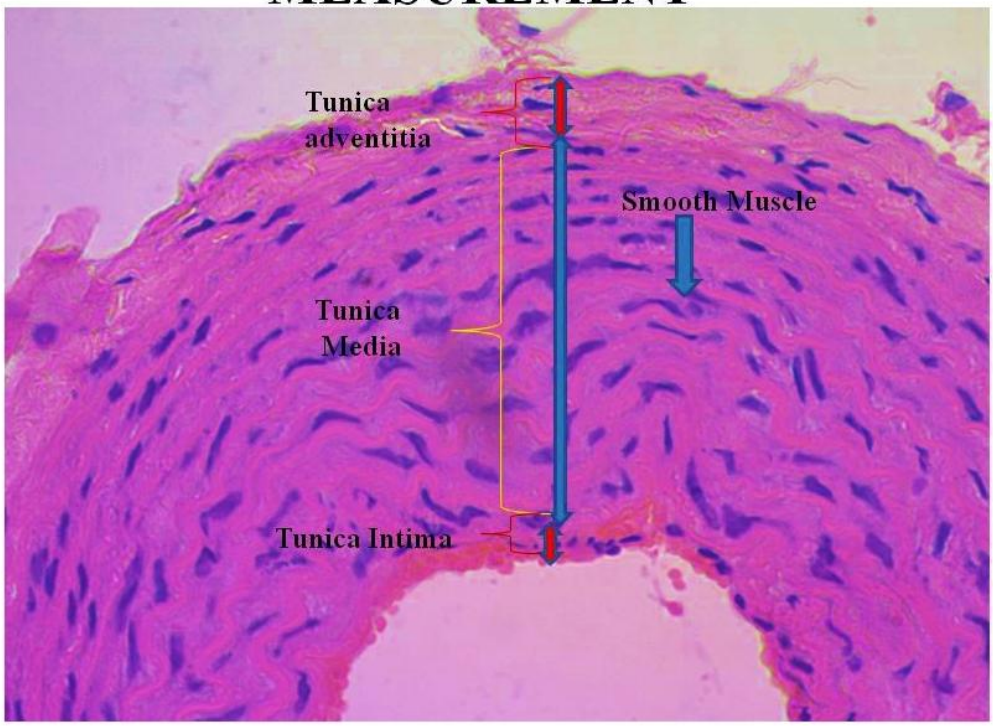
Parameters	**Group1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Anova**	
						F value	**P value**
Tunica Intima µm	35.8±2.1	29±4.2[a]	37.8±3.8[b]	37.5±4.2[b]	38±4.7[b]	7.28	0.002
Tunica Media µm	196±47	191±32	211±41	193±48	211±55.9	0.26	0.89

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5.(*p ≤0.05).

Table 8. shows effect of ethanolic extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* on Elastic arterial thickness.

The result of group 2 rats fed with high fat diet indicates significant decrease of tunica intima thickness in Elastic artery as compared group 1normal/Isocaloric diet fed rats. Whereas, both *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* supplemented groups shows near normal elastic artery thickness. Interestingly tunica media thickness in group 2 rats did not show any significant alteration in thickness.

ELASTIC ARTERY WALL THICKNESS MEASUREMENT



VEHEROFFS STAIN ELASTIC ARTERY

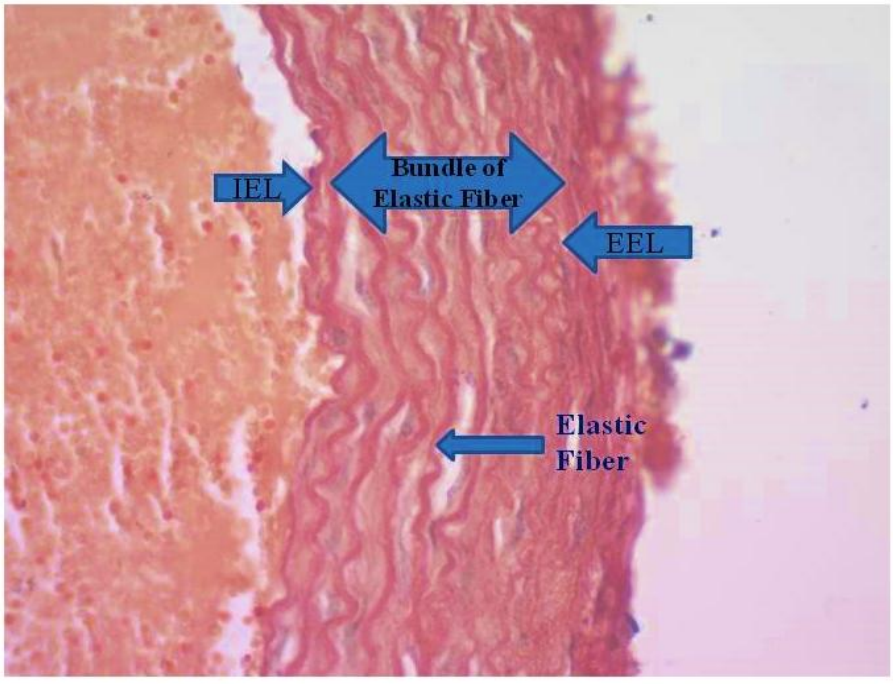


TABLE 9. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Morphometry of Elastic Arterial Lumen

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	Anova	
						F value	P value
Antiro-Post µm	765.9±76	571.9±42[a]	777.3±37[b]	777.5±27[b]	802±15[b]	26.6	0.000
Transverse µm	212.5±109	168.2±60[a]	217.6±40[b]	221.8±40[b]	216.8±103[b]	53.7	0.000
Arterial Lumen μm^3	722.7±28	577.3±27[a]	788.7±15[a,b]	775.3±36[a,b]	777.2±27[a,b]	59.4	0.000

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5.(*p ≤0.05).

Table 9. shows effect of ethanolic extract of *Terminalia Arjuna (Arjuna)* & *Emblica Officinalis (Amla)* on Elastic arterial lumen morphology.

Results clearly indicate significantly decrease of luminal Anteroposterior diameter, transverse diameter and area of arterial lumen in group 2rats fed with high (Hyperlipidemic diet) fat diet. Supplementation of drug 1 is *Terminalia Arjuna (Arjuna)* & *Emblica Officinalis (Amla)* and combination of both show significant improvement of liman area, anteroposterior and transverse diameter.

TABLE 10. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Muscular Artery Thickness

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	Anova	
						F value	P value
Tunica Intima µm	25.4±7.3	25.8±5.3	31.2±3,7	24.7±7.9	26.2±4.1	1.13	0.36
Tunica Media µm	139±58	99±17	167±70	137±60	131.4±30.5	1.32	0.28

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5.(*p ≤0.05).

Table10. Depicts Effect of ethanolic extract of T*erminalia Arjuna* (Arjuna) & E*mblica Officinalis* (Amla) on muscular artery thickness, results shows insignificant changes in tunica intima and tunica media thickness in group 2 hyperlipidemic rats

TABLE 11. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Coronary Arterial Wall Thickness Measurement

Parameters	**Group1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Anova**	
						F value	**P value**
Coronary Artery Thickness µm	192.4±6.7	251.9±18.4[a]	199.4±16.6[b]	193.8±9.8[b]	212.2±4.9[b]	23.32	0.000

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5. (*p ≤0.05).

Table11. Effect of ethanolic extract of *Terminalia Arjuna (Arjuna)* & *Emblica Officinalis (Amla)* on coronary arterial wall thickness measurement. Shows significant increase of coronary arterial wall thickness in group 2 rats compared its control group 1.

Supplementation of drug in group 3 fed with high fat diet treated with *Terminalia Arjuna (Arjuna)*, group 4 fed with high fat diet treated with *Emblica Officinalis (Amla)*, group 5 fed with high fat diet treated with both the drug *Terminalia Arjuna (Arjuna)* & *Emblica Officinalis (Amla)* shows remarkable improvement in coronary arterial wall thickness.

CORONARY ARTERY WALL THICKNESS

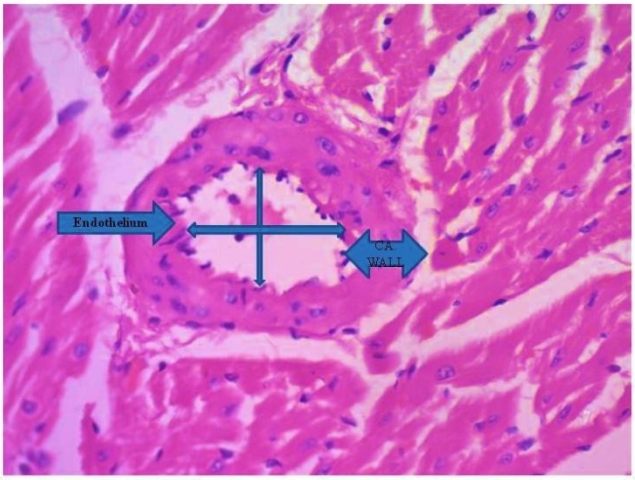


TABLE12. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Analysis of Coronary Arterial Lumen Measurement

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	Anova	
						F value	P value
Antiro-Post µm	793.9±57.7	569±117	638.3±194	726.7±136	1106 ±100 [b,c,d]	7.76	0.004
Transverse µm	591.8±35	499.1±45	1208.4±98.1 [a,b]	1027.7±166 [a,b]	1588±260 [a,b,c]	27.8	0.000
Arterial Lumen µm^3	366.9±45	268.1±46 [b]	496±29.4 [b]	653±124 [a,b]	741.8±56 [a,b,c]	24.08	0.000

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5. (*p ≤0.05).

Table12. Effect of ethanolic extract of *Terminalia Arjuna (Arjuna)* & *Emblica Officinalis (Amla)* on analysis of coronary arterial lumen measurement depicts a significant narrowing of vascular integrity by reducing Anteroposterior diameter, transverse diameter and compromising area of coronary arterial lumen. Interestingly, group 3, 4 and 5 showed amelioratic effect on lipid induced impact on coronary arterial vascular integrity.

TABLE 13 Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Normalized Wall Index (NWI)

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	Anova	
						F value	P value
Normalized wall Index (NWI)	0.30 ± 0.01 [b]	0.44 ±0.03 [a]	0.30 ±0.02 [b]	0.31 ± 0.02 [b]	0.31 ± 0.26 [b]	37.4	0.000

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5. (*p ≤0.05).

Table13. Effect of ethanolic extract of *Terminalia Arjuna (Arjuna)* & *Emblica Officinalis (Amla)* on normalized wall index (NWI) shows the results clearly indicate significant in group 2 rats fed with hyperlipidemic diet.

In case of supplementation of drug 1 (Group 3), drug 2 (Group 4) and Drug 1&2 (Group 5) normalized wall index had brought back to near control group.

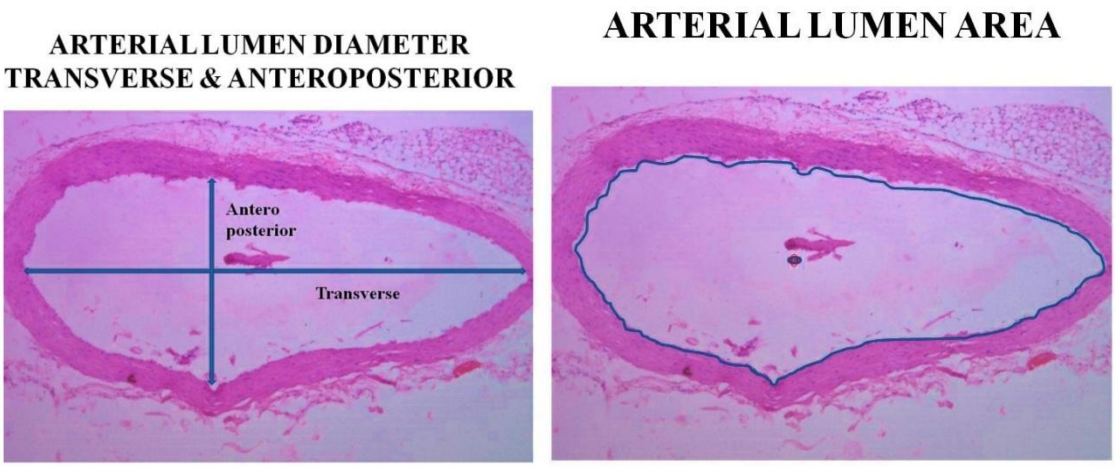


TABLE 14. Effect of Ethanolic Extract of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla*) on Serum Nitric Oxide (NO)

Parameters	Group1	Group 2	Group 3	Group 4	Group 5	Anova	
						F value	P value
NO	73.8± 22.6	87.1±19	69±16.2	62.8±27.9	78.2±22.7	1.04	0.4

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups. a depicts comparision with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 5.(* ≤0.05).

Table 14. Effect of ethanolic extract of *Terminalia Arjuna (Arjuna)* & *Emblica Officinalis (Amla)* on serum nitric oxide (No). We observed decreased level of NO in group 3 and 4 compared to group 1 although it was non -significant. There was an insignificant increase in NO levels of group 5 compared to group 1 ($p \leq 0.05$).

CHAPTER 6

DISCUSSION

DISCUSSION

Phytochemistry:

Phytochemical analysis shows both the drugs *Terminalia Arjuna (Arjuna) and Embilica Officinalis (Amla)* does not have any toxic ingredients.

Gravimetry:

Increased body weight levels in hyperlipidemic diet clearly indicate lipid induced additional fat synthesis. The supplementation of *Terminalia Arjuna (Arjuna) and Emblica Officinalis* (Amla) shows protection against lipid induced lipogenesis.

Haematology:

Unchanged hematological profile in all the groups indicated both these two drugs *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* are not having any adverse effect on hematopiosis. It also indicates *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* are safe to consume as per hematology concerned. Our findings are corporate with Arumugam Shivagurunathan et al[1].

GENERAL

Lipid Profile:

Our result from lipid profile clearly indicates dyslipidemia in case of high lipid fed diets rats in group2, but surprisingly, both the drugs *Terminalia Arjuna (Arjuna) and Emblica Officinalis* (Amla) shown a protective action against lipid induced hyperlipidemia. Possibly, presence of high flavonoids and polythenol compounds with its antioxidant property act as hyperlipidemic agent[2].

Feeding of high fat diet (group 2) may also result in excess hepatic triglycerides due to increased synthesis of triglycerides and increased de novo lipogenesis (DNL). The

supplementation of *Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla)* may induce an inhibitory effect on intestinal dietary fat absorption that leads to decrease triglyceride accumulation[3]. In addition, it may also possibly due to ability of greater functioning insulin to stimulate glucose transport mechanism in adipocyte and in skeletal muscle which is impaired due to hyperlipidemia resulting in insulin resistance. Impairment of insulin sensitivity or possibly insulin resistance leads to dyslipidemia[4].

Hence, in our study the presence of beneficial phytoconstituents of *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* inhibit effectively fat accumulation and ameliorate dyslipidemia in high lipid fed rats.

Liver function test:

Liver function tests clearly indicate no such changes of any parameters that is serum bilirubin, direct and indirect serum bilirubin, SGOT, SGPT, serum protein and alkaline phosphatase indicate the high lipid fat diet did not alter hepatocellular function in our study.

Both these two drugs *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* cause no alteration in liver function test which indicates both these two drugs *Terminalia Arjuna (Arjuna)* and *Embilica Officinalis (Amla)* are non toxic to Liver function tests and may be used as safe ingredients.

Usually most of the drugs based on pharmaceutical substances cause contraindication effects on hepatic function test. Hence major safety concerned any drug use in any research protocol is essential. Hence, both *Terminalia Arjuna (Arjuna)* and *Embilica Officinalis (Amla)* may be considered as safe as per Liver function concerned[5].

Serum electrolytes:

No change in levels of electrolytes in our study indicates neither hyperlipidemic diet nor *Terminalia Arjuna* (Arjuna) and *Embilica Officinalis (Amla)* any influences on electrolytes and its homeostasis

Glucose regulation:

No change in Random Blood Sugar level in any groups in our study indicates unimpaired glucose homeostasis among all the groups, including the group of rats fed with hyperlipidemic diet.

SPECIAL

Histology of Atrium:

Normally, high fat diet causes remodeling the Atrial structure, but it is not confirmed whether high fat diet induces changes in histology of Atrial texture. In our study, we did not find any change in Atrial histological architecture in high fat diet fed rats.

Histological architecture of atrium of group 2 findings of high fat diet induced rats clearly indicate that short term, high fat diet feeding may not alter the Atrial wall architecture in our study.

Normally, excess lipid may stimulate mitochondria overload and activate myocardial molecular intimal cardiac remodeling.

Ventricle:

In our study on ventricular histology did not show any significant change of ventricular histology in group 2 rats, except we observed in few rats myocardium containing coronary artery showing an early changes of atherosclerosis which indicate minimal cardiac metabolic disturbances by high dietary fat.

Elastic artery:

We observe that in the endothelial layer of elastic artery shows an early change of atherosclerotic plaque and even we observe that there is a mild alteration in the arterial wall histology. These alterations may include arterial wall modification with component changes in the arterial wall and same in stiffer aorta. This study has been conducted on short term fat diet basis.

In our study early atherosclerotic changes in group 2 rats reflect less elasticity in arterial wall which may lead to increase in peripheral resistance and blood pressure[6].

It has been already reported that elastic artery changes of tunica intima and tunica media increase with high fat diet and it leads to increase arterial stiffness from small arteries to large arteries[7].

Increase thickness of elastic artery; tunica intima and tunica media are partly due to increase in smooth muscle cells. It was reported that aortic intima and media thickness was an earlier marker of preclinical atherosclerosis, which had been observed in group 2 in our study[8].

The function of elastic fibers in the arterial wall is the maintenance of tension without constant expenditure of energy. According to Burton the arterial tension has a correlation to the amount of elastic tissue present in the vessel wall. Since coronary arteries arise from the root of aorta, they are subjected to maximum pressure during each cardiac cycle and hence have abundant elastic fibers to maintain arterial tension[9].

From table no 8 and 9 we have clearly found decreased lumen, increased arterial wall thickness which again bring back to the normal in case of group 3, 4 and 5 with supplementation of drugs *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla).*

The result indicates loss of arterial compliance with possible stiffening accompanied by histological modification of arterial wall due to high fat diet. Perhaps the internal elastic lamina or media component might be enriching fibers components such as collagen and elastin. The high fat diet induces changes in this vascular integrity and induces loss of elasticity. This increase in collagen was partly an addition to the bulk of the media but in later life it was partly at the expense of smooth muscle[9] Thus, alteration of mechanical priority which may lead to severe cardiovascular dysfunction[10].

Although the mechanism is reinitiating arterial remodeling in high dietary lipid which induce metabolic disorder but earlier studies have reported that high lipid causes concomitant reduction in arterial luminal diameter[11].

In our study the supplementation of *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* and both drugs groups, show a significant improvement in lumen diameter accompanied by a significant decrease of arterial wall thickness in rats fed with high lipid diet.

These results clearly show that improvement of elastic arterial property by treatment with *Terminalia Arjuna (Arjuna)* in group 3 and *Emblica Officinalis (Amla) in group 4* and both the drug *Terminalia Arjuna (Arjuna)* group and *Emblica Officinalis (Amla)* treated group 5.

Muscular Artery:

High lipid fat diet did not show any alteration of a muscular artery of the table shows (Table No 10) at any level of tunica adventitia, tunica media or tunica intima. It clearly indicates the short term effect of high lipid diet apparently does not have any adverse impact on muscular arterial integrity.

Coronary artery:

Smoking, hypertension, diabetes, fibrinogen, and low density lipoprotein cholesterol (LDL cholesterol) are widely accepted coronary heart disease (CHD) risk factors. These risk factors are also associated with preclinical atherosclerosis, generally measured as the intima-media thickness (IMT)[12].

In our observation of coronary arterial wall and lumen integrity; changes have shown in the lumen area as an early change of atherosclerosis in tunica intima as well as tunica media in high fat fed rats.

Atherosclerosis is common condition which leads to inflammatory status of the micro vessels leading to the development of ischemic heart diseases or cerebrovascular disease or peripheral vascular disease. One of the common risk factor for atherosclerosis is high dietary lipid which induces pathophysiological vascular phenotypic alterations[13].

Atherosclerosis is a disease of the tunica intima. The tunica intimal layer is separated from the tunica media layer by the internal elastic lamina (membrane) (internal elastic lamina, elastica interna), which is a fenestrated sheet of elastic tissue[14].

The tunica media consists of multiple layers of smooth muscle cells and connective tissue (elastic fibers, collagen, proteoglycans). The amount of elastic tissue is less and the number of smooth muscle cells is greater in the epicardial coronary arteries than in other elastic vessels[15].

The tunica media consists of up to 40 layers of circumferentially or helically oriented smooth muscles. The normal tunica media ranges in thickness from 125-350 pm (average 200 pm)[16].

Tunica media thickness underlying diseased intima (atherosclerotic plaque) is considerably thinner, ranging from 16 to 190 pm (mean 80 pm)[16]. The smooth muscle cells are embedded in a glycoprotein mix that stains heavily with the periodic acid-Schiff reactions (being PAS positive).

Collagen and elastic fibers are also present in this layer. The tunica media layer is separated from the tunica adventitial layer by the external elastic lamina (membrane) (external elastic lamina, elastica externa). The external elastic membrane is composed of interrupted layers of elastin and is considerably thinner than the internal elastic membrane[14]

The increase in tunica intima was found to be the basic pathological change which ultimately progress to atherosclerosis[9].

One of the most important initial events in the development of atherosclerosis is accumulation of cells containing excess lipid within the arterial wall which are mostly macrophages and transformed monocytes, those engulfs oxidized LDL to become foam cell of fat laden macrophages[17].

The tunica intima and greater part of the tunica media lack capillaries and receive nutrition by diffusion; hence oxygen and other nutrients including soluble blood lipids must diffuse from lumen through the intercellular substance of the intima and most of the media. With abnormal increase in intimal thickening this diffusion mechanism gets very much deranged resulting in insufficient oxygen tension in tissues of arterial wall[9].

Internal elastic lamina showed splitting, fraying, fragmentation and reduplication in various age groups[9].

Most of the drugs against atherosclerosis are basically the inhibitor of HMG CoA reductase which is important for regulating relimiting step for cholesterol biosynthesis. The substantially important of coronary arterial wall thickness and lumen diameter and lumen area after treatment with these two drugs *Terminalia Arjuna* (Arjuna) and *Embilica Officinalis (Amla)* might be due to their potential impact of HMG CoA reductase on pathways.

In our study increased coronary arterial wall thickness with concomitant reduction of the coronary arterial wall area (change of Antiroposterior and transverse diameter) also in group 2 rats attributed a possibility of high oxidized LDL, oxidative stress that results from ROS leads to transform monocytes to macrophage and further develop foam cell, which fills subintimal layer and form fatty streak in the coronary artery[18].

The fat induced injury on subintimal layer may also initiate various cytokines and growth factors which stimulate migration and proliferation of smooth muscle cell that became intermix with the area of inflammation to form intermediary lesion. If these response continue further causes increase in thickness of coronary arterial wall with compensatory slow dilatation[19].

It has been noticed that stimulation of proinflammatory markers, cytokines, chemokines activate atherosclerotic plaque. The antioxidant effect of *Terminalia Arjuna* (Arjuna) and *Embilica Officinalis (amla)* observed in present study of groups 3, 4 and 5 may be mediated by protecting LDL oxidation which is one of the early changes of atherosclerosis[20].

Thickening of coronary arterial wall definitely compromise coronary arterial lumen diameter and surface area which we have noticed in our observation in case of high lipid fed rats. There is reduction in coronary arterial diameter, structural area with increase in wall thickness.

Normalized wall index:

It has been reported that normalized wall index indicator of cardiovascular diseases of and mean wall index might be useful to assess the atherosclerotic disease burden. Although in our study mean lumen area showed a significant change in group 2 as compared to normal. But many other cardiovascular diseases lumen area does not show any changes. Hence simply assessing lumen or area may be considered as less sensitive marker of the atherosclerotic disease burden than normalized wall index[21].

Decrease in group 2 normalised wall index also indicate negative remodeling and in our study supplementation of drug *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis(Amla)* both the drugs shows a remarkable improvement in normalized wall index which may be considered as passive indicator for coronary arterial structural integrity.

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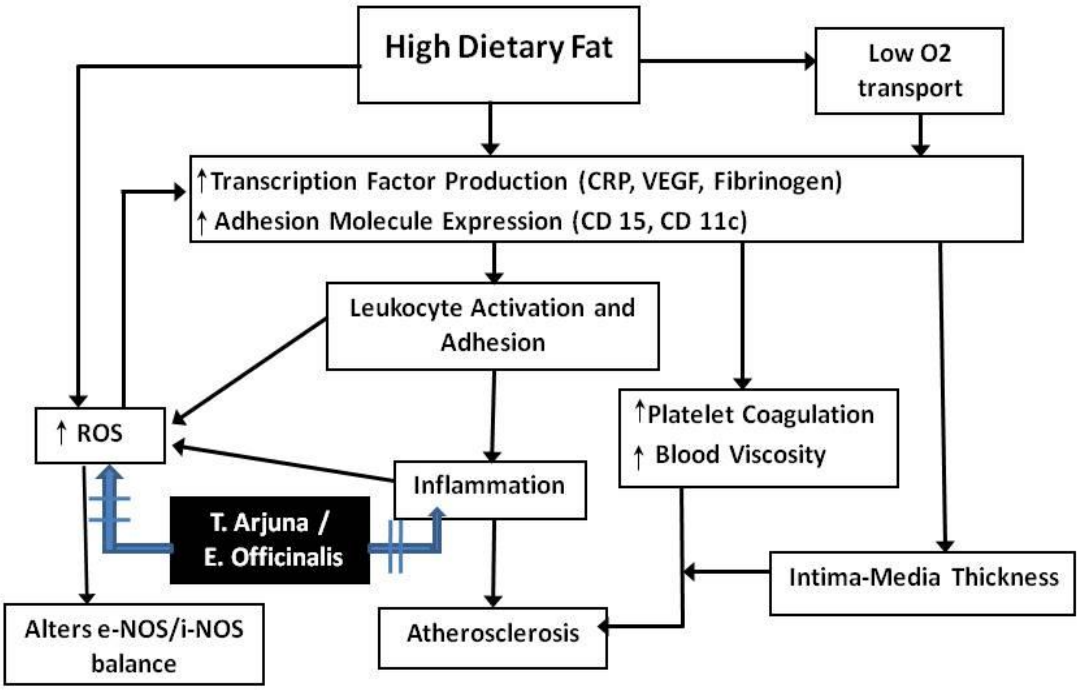
SUMMARY CONCLUSION

SUMMARY CONCLUSION:

The entire work on the effect of high lipid diet (30%) on general and Cardiovascular impact in albino Wister rats with special reference to drug 1 Terminalia Arjuna (Arjuna), drug 2 Emblica Officinalis (Amla) and Drug 3 both Terminalia Arjuna (Arjuna) and Emblica Officinalis (Amla) may be summarized as following figure



- High fat diet, even alters Vascular properties in adult male albino Wistar rats of approximately 200 days (Average age, middle age).
- The alteration of vascular integrity is mainly reflected by histopathological changes in arterial tissues, elastic artery, Coronary artery without altering the nitric oxide status in rats with high fat diet.

- The early atherosclerotic changes in coronary artery in high fat fed rat perhaps due to age associated vascular disorders

- Both *Terminalia Arjuna & Emblica Officinalis* are found to be cardioprotective to some extent without influencing any metabolic impairment of liver

SUMMARY

- 10% of additional fat to Iso-caloric diet for short term duration of time on 200 days old rats develops early alteration in vascular integrity reflected by the mild formation of atherosclerosis plaque in coronary vessels along with increase wall thickness.

- This is further correlated that aortic wall in which significant structural changes by observing elastic artery, muscular artery at the level of tunica media the most.

- The adverse changes on vascular integrity were partial recovered by *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* perhaps through free radical scavenging properties at least in histopathological point of view

LIMITATION:

Certain molecular markers for vascular integrity like nos3 gene expression Akt gene expression were evaluated in this study, hence the molecular mechanism behind these two drugs Terminalia Arjuna (Arjuna) and Emblica Officinalis to regulate hyperlipidemia induced cardiovascular alteration could not be explored.

FUTURE DIRECTION:

1. Molecular markers to be evaluated
2. High dietary lipids induce through study on oxidative and nitrosative stress to be done.
3. Active compounds of both the drugs further evaluated by HPLC method.

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Original Article

EFFECT OF ETHANOLIC EXTRACT OF *TERMINALIA ARJUNA* ON LIVER FUNCTIONS AND HISTOPATHOLOGY OF LIVER IN ALBINO RATS FED WITH HYPERLIPIDEMIC DIET

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ABSTRACT

Objective: The aim of the present study was to assess the effect of Ethanolic extract of *Terminalia Arjuna* on Liver functions, Lipid profile and histopathology of liver of albino rats fed with Hyperlipidemic diet.

Methods: Extraction of *Terminalia arjuna* bark by Soxhlet apparatus using 99% ethanol at 60 ° temp for 22 h and Phytochemical analysis was done. Group 1 served as normal control. Group 2 Fed with Isocaloric diet. Group 3 Fed with Hyperlipidimic diet. Group 4 Hyperlipidemic diet 21 d+*Terminalia arjuna* 21 d.

Dose of Ethonolic extract of *Terminalia arjuna*: (500 mg/kg Body weight daily).

Results: %body weight gain and hepatosomatic index were significantly improved in hyper lipidemic rats treated with *Terminalia arjuna*. There was significant improvement in markers of liver functions. Liver shown microvescicular and macrovesicular fatty changes in hyper lipidemic rats and normal Hepatocytes in Hyperlipidimic rats treated with *Terminalia arjuna.*

Conclusion: It can be summarized that *Terminalia arjuna* is good, natural therapeutics in hyperlipidemia and liver disorders.

Keywords: *Terminalia arjuna,* Hyperlipidimic diet, Histopathology of Fatty Liver, LFT and hepatosomatic Index.

INTRODUCTION

This is the speed changing world of extreme disparity like over nutrition and starvation, lifestyle changes and its related diseases, development and environmental degradation. The modern system of medicine is no exception. It cures one hand and trigger side effects on the other [1].

There is increasing demand of people towards an Ayurvedic system of medicines which shows reduced adverse effects on health.

Among many precious herbal drugs *Terminalia arjuna* holds the pride place in the reference of such medicinal value. *Terminalia arjuna* is a deciduous and green tree belongs to *Combretaceae family*, also known as *Arjuna or Arjun tree. Terminalia arjuna* tree is about 60 to 80 feet in height it is found in Indo-sub Himalayan tracts of Uttar Pradesh, southern Bihar, Barma, Madhya Pradesh and Duccan region. It grows almost in all types of soils, but prefers humid, fertile loam and lethargic soils.

The bark gets flaked off itself in the month of April-May[2] Its stem bark has active principles like glycosides, flavonoids, tannins and minerals [3]. Flavonoids acts like antioxidants, anti-inflammatory and lipid lowering effect where as glycosides are cardio tonic. It also shows hepato-protective effect [4].

The liver is the primary organ which plays an important role in metabolic and excretion and maintains homeostasis of the body[5] Hyperlipidemia is greatest risk factors for prevalence and severity of coronery heart diseases [6] Hyperlipidemia is also one of the major causes of liver injury. Management of liver diseases still challenges to the modern system of medicine.

Terminalia Arjuna is capable of protecting the liver against hyperlipidaemia, oxidative stress and/or toxin effects [7].

Hence the present study was aimed to assess the effect of Ethanolic extract of *Terminalia arjuna* on liver in albino rats fed with hyperlipidemic diet. The effects were evaluated by measuring the levels of the serum marker enzymes followed by liver histo pathology.

MATERIALS AND METHODS

Materials: Fresh, bark of *Terminalia arjuna* was procured from the herbal garden of Ayurvedic medical college, during the months of Nov–Dec 2012 identified and authenticated by Department of Botany KCP Science College Bijapur

Extraction of drug

Ethanolic extract preparation: 250 gms of the powder of the dry bark of *Terminalia arjuna* were extracted with 99% ethanol using a Soxhlet apparatus at a temperature below 60 ˚C for 22 h. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder [8].

Phytochemical screening

Terminalia arjuna was screened for the presence of phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrates by using standard protocols [9].

Experimental animals

Albino Wistar rats weighing 160 to 250 gms were obtained from animal house of Shri B M Patil Medical College Hospital and Research Centre, Bijapur. All the four group animals were acclimatized for 7 d to the laboratory conditions at 22-24 ºC and maintained 12 HR. Light/dark cycle all the experimental procedures were performed in accordance with the approval of the Institutional Animal Ethics Committee (IAEC) of Shri B M Patil Medical college Hospital and Research Centre, Bijapur. All care has taken on animals during experimental as well as at the time sacrificed as per the guidelines of ICMR on animal research 2006

Experimental protocol

All the rats were divided into following four groups with 6 rats in each group. Group-I, Fed with water and ad libitum serve as a control, the Group II Fed with Isocaloric diet for 42 d Group-III Fed with high fat diet 42 d, the Group IV Fed with high fat diet and Ethanolic extract of *Terminalia arjuna* (21 d high fat diet+21 d with

Ethonolic extract of *Terminalia arjuna*). It was given daily 500 mg/kg Body Weight, I. P [10].

Preparation of isocaloric diet

For 1 kg of diet, 180 gm of casein, 620 gm of carbohydrate, 200 gm of fat and 1% of multivitamin and 2% NaCl was taken [11].

Preparation of hyperlipidemic diet

For 1 kg of diet, 180 gm of casein, 520 gm of carbohydrate, 300 gm of fat and 1% of multivitamin and 2% NaCl was taken [11].

Sample collection and tissue collection

All the four group animals were sacrificed by cervical dislocation at the end of the last dose after an overnight fast. After heart puncture blood was collected in normal tubes for the separation of serum,

Tissue collection for histopathology: liver was isolated immediately and fixed in 10% neutral buffered formalin solution for 24 h. The fixed tissues were processed routinely, and then embedded in paraffin, sectioned into 4–5 µm thickness, deparaffinized, and rehydrated using standard techniques. The extent of Hyperlipidimic diet-induced necrosis and steatosis was evaluated by assessing morphological changes in liver sections stained with hematoxylin and eosin (HandE), using standard techniques.

Gravimetry

Estimation Body Weight and Hepato-somatic Index of Albino Wistar rats:

The body weight of all rats was recorded at the beginning of the experiment (day 1), treatment with an Ethanolic extract of *Terminalia arjuna* (21st day) and on the day of sacrifice (42nd day). Liver weight was measured to the nearest of 0.1 mg in a single pan balance (Digital weighing machine). Further, we calculated Hepato-somatic index by the formula liver weight/total body weight.

Biochemical analysis

In biochemical analysis, we estimated lipid profile and liver function test

Estimation lipid profile

Serum triglycerides (TG), Total Cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were analysed by the MESPA automated Analyzer.

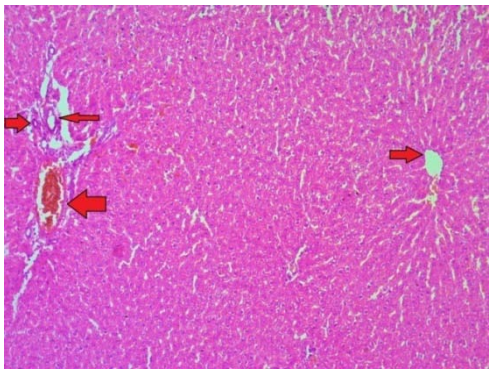

Fig. 1: G1,10X. H and E stain showing normal hepatocytes in lobular pattern

Estimation of liver function tests

Serum Bilirubin, total, Serum Glutamic Oxalocetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Serum Protein, Serum bilirubin, Serum albumin, Serum A/G Ratio and Serum Alkaline Phosphatase (ALP) analysed by Meril diagnostic Kit Method.

Statistical analysis

Values are expressed as mean±SD. To determine the significance of intergroup differences, One Way ANOVA followed by 'Post Hoc t tests' by SPSS software was done. ₱0.05 was considered statistically significant.

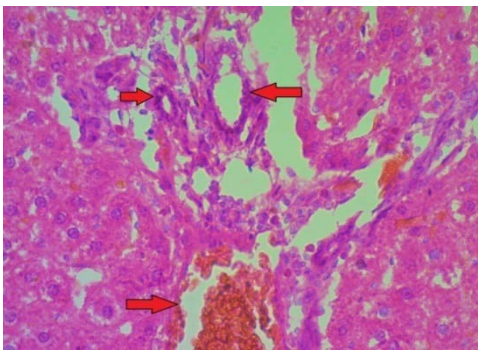

Fig. 2: G140X. H and E stain showing normal architecture of liver with portal tried (Bile Duct, Hepatic Artery, and Vein)

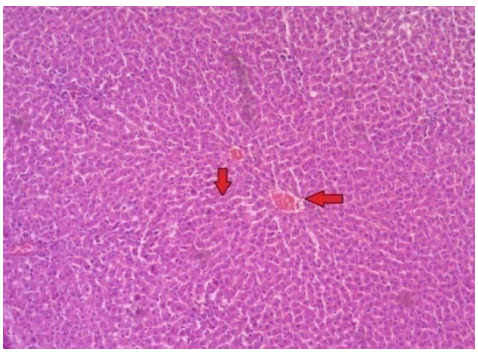

Fig. 3: G2 10X H and E stain showing the normal architecture of the Liver with Central vein surrounded by Hepatocytes and intervening Sinusoids

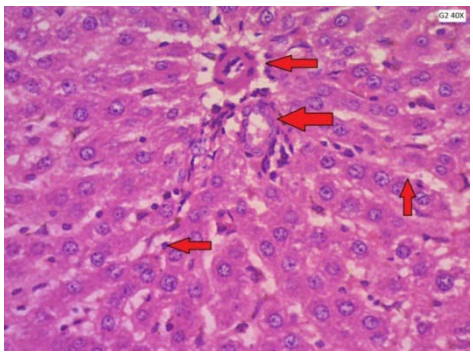

Fig. 4: G2 40X H and E stain showing the normal architecture of the liver with central vein surrounded by hepatocytes and intervening sinusoids

RESULTS

We observed a significant increase in levels of TG and VLDL in group 3 (Hyperlipidemic diet fed rats) compared to group 1 (Normal control rats). Group 4 (hyperlipidemic rats treated with ethanolic extract of *Terminalia arjuna*) rats showed a significant increase in levels of TC and LDL compared to Group 1, 2 and 3 respectively. HDL levels showed a significant increase in group 3 (Hyperlipidemic diet fed rats) compared to groups 1 and 2 respectively. We also observed significant decrease in LDL levels in group 3(Hyperlipidemic diet fed rats) compared to groups 1 and 2 respectively

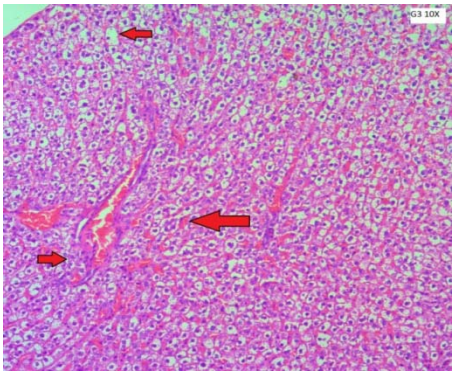

Fig. 5: G3 10X HandE stain. architecture of liver showing prominent microvesicular and macrovesicular fatty change

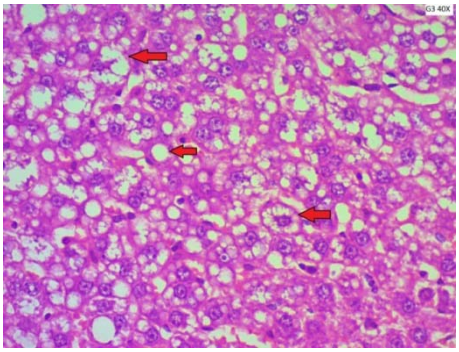

Fig. 6: G3 40X HandE stain architecture of liver showing prominent microvescicular and macrovescicular fatty change

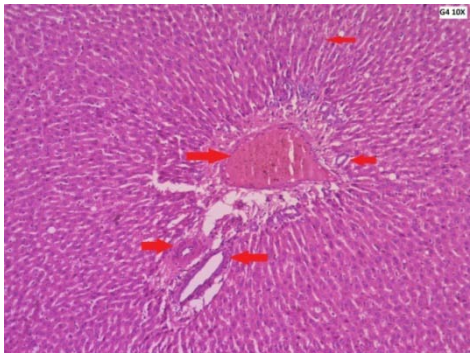

Fig. 7: G4 10X HandE stain architecture of liver showing prominent hapatocytes separated by dilated sinusoids

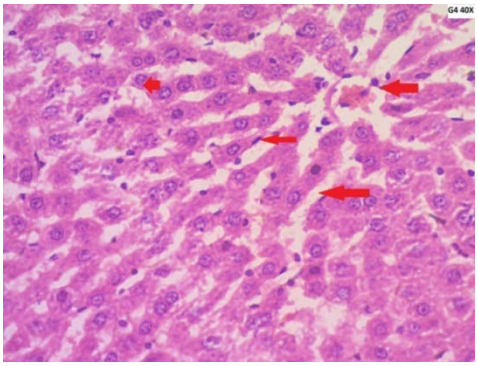

Fig. 8: G4 40X HandE stain architecture of liver showing prominent hapatocytes separated by dilated sinusoids

Table 1: Effect of ethanolic extracts *Terminalia arjuna* on lipid profile

Parameters	Group 1	Group 2	Group 3	Group 4	ANOVA	
					F value	P value
TG mg/dl	104.5±13.8	134.3±25.1	206.5±90.7[a]	129±32	4.58	0.013
TC mg/dl	130.6±13.6	122.8±16	123.1±17.5	185±17[a,b,c]	20.48	0.000
HDL mg/dl	30.3±1.8	30±1.9	41.8±14[a,b]	36.8±2.5	3.64	0.03
LDL mg/dl	80.6±12.5	64.7±18.5	33±15.8[a,b]	122.3±14[a,b,c]	34.7	0.000
VLDL mg/dl	22.7±4.2	28±7.1	40±18[a,]	25.8±6.4	3.0	0.05

Values are expressed as mean±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3. (* ≤0. 05). Group 1 is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is a hyperlipidemic+Ethanolic extract of *Terminalia Arjuna.*

Table 2: Effect of ethanolic extracts *Terminalia arjuna* on markers of liver function

Parameters	Group 1	Group 2	Group 3	Group 4	ANOVA	
					F value	P value
Serum Bilirubin mg%	0.6±0.1	0.6±0.1	0.7±0.1	0.8±0.1	2.11	0.13
SGOT U/l	76.6±6.5	51.3±8[a]	70±28[b]	50±8.1[b]	3.38	0.000*
SGPT U/l	59.5±19	47.1±5.6[a]	64.5±12[b]	46±3.6[b]	3.03	0.000*
Serum Protein gm/dl	5.7±0.2	6±0.3	5.7±0.2	5.6±0.2	1.38	0.16
Serum Alb gm/dl	2.9±0.1	3.4±0.3[a]	3±0.1[b]	2.8±0.1[b]	6.99	0.002*
Serum A/G gm/dl	1±0.1	1.3±0.2[a]	1±0.1	0.98±0.04[b]	5.21	0.008*
Serum ALP U/l	153±16	174±26	187±50	161±18	1.4	0.27

Values are expressed as mean±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3. (* ≤0. 05). The group 1 is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is a hyperlipidemic+Ethanolic extract of *Terminalia Arjuna.*

We observed significant higher levels of SGPT and SGOT in group 3 compared to group 2(*P<0.05). Similarly, we observed significant changes for SGPT and SGOT in group 2 and group 4.

There was significantly higher values of Serum albumin and the Serum A/G ratio in group 2 compared to group 1(*p<0.05). Group 4 has shown significant lesser values of Serum albumin and the Serum A/G ratio compared to group 2 (*p<0.05).

We also observed no significant differences for serum bilirubin, serum protein and serum ALP among all groups.

We observed no significant differences for % body weight gain among all groups at 21st day.

Similarly, we observed significantly decreased in % body weight in group 4 compared to group 1, 2 and 3 (*p<0.05).

Table 3: Effect of ethanolic extracts *Terminalia arjuna* on % body weight gain

% Body weight gain	Group 1	Group 2	Group 3	Group 4	ANOVA	
					F value	p value
At 21st day	20.6±1.2	22.4±1.7	20.9±2	21.5±1.9	1.27	0.30
At 42nd day	17.5±2	18.6±1.3	19.1±2	14.4±1.3[a,b,c]	8.68	0.001*

Values are expressed as mean±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3. (* ≤0. 05). Group I is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is a hyperlipidemic+Ethanolic extract of *Terminalia Arjuna*

Table 4: Effect of ethanolic extracts *Terminalia arjuna* on hepato-somatic index

Oregano somatic index	Group 1	Group 2	Group 3	Group 4	ANOVA	
					F value	p value
Hepato-Somatic Index	0.03±.003	0.026±.002[a]	0.02±.003[a]	0.02±0.002 [c]	14.307	0.000

Values are expressed as mean±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3. (* ≤0. 05). Group I is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is a hyperlipidemic+Ethanolic extract of *Terminalia Arjuna*

We observed the significant decrease in the hepatic-somatic index in group 2, 3 compared to group 1. Also significant difference was seen in between group 3 and group 4 (*p<0.05).

Effect of *Terminalia Arjuna* (Ethanolic Extract) on liver histopathology

Fig. 1 and 2, G1 (Group 1) 10X and 40X HandE stain histopathology section of liver shown normal hepatic architecture compressed of hepatic lobules formed by the central vein and the cords of hepatocytes with indistinct sinusoidal dilatation in fig. 3,4,7and8. G2and4 (Group 2and4)10X and 40X HandE stain shown prominent sinusoidal dilatation.

Whereas G3 (Group 3)10X and 40X HandE stain histopathology shown lobular architecture of the liver with enlarged hepatocytes containing microvescicular and macrovesicular fatty changes with sinusoidal congestion.

G4 (group 4) Bark extract of *Terminalia arjuna* (500 mg/Kg body weight) treated rats histopathology section of liver shown normal hepatic architecture

DISCUSSION

In the present study Ethanolic extract of *Terminalia arjuna* bark was tested for their hepato-protective activity in albino rats fed with hyperlipidemic diet. The degree of protection was assessed using markers of liver functions like serum albumin, serum globulin, serum A/G, SGPT, SGOT and serum ALP along with histopathological study.

The peculiar sign of hepatic damage is leakage of cellular enzymes into the serum. The SGPT, SGOT and ALP are important cellular enzymes [12].

When there is hepatopathy, these enzymes leak into the blood stream from the cytoplasm with an extent of liver damage. AST, ALT and bilirubin levels are commonly measured as an indication of hepatocellular integrity. ALT is frequently used as the biochemical parameter to asses hepatic injury[Anitha] [13]In the present study, increased levels of serum SGPT, SGOT and ALP in hyperlipidemic rats showed the damage of liver tissue. The decreased level of serum SGOT, SGPT and ALP in hyperlipidemic rats treated with *Terminalia arjuna* showed repair of hepatic cells by restoring cell permeability. Similar findings were observed in Hardik Soni *et al.* study [9].

In the present study, we observed a nonsignificant increase in bilirubin level in hyperlipidemic rats treated with *Terminalia arjuna* compared to hyperlipidemic rats. In contrast, P Doorika *et al.* reported significant decrease in bilirubin levels in rats treated with *Terminalia arjuna* [4].

The liver plays an important role in the synthesis of protein like Albumin [15].

Akanksha P S, *et al.* reported decreasing level of albumin, total protein level and damage to the normal architecture of the liver. Similar findings were observed in our study [5].

We observed marked structural alteration, i.e. microvescicular and macrovesicular fatty changes in histopathology of liver of rats fed with high fat diet. Hyper lipidemic rats treated with ethanolic extract of *Terminalia arjuna* is shown normal architecture of liver histology. Ragavan, B., *et al.* reported in their study, high fat diet induced rat treated with *Terminalia arjuna* bark extract shown to partially reverse the damage (Fatty changes) [16].

CONCLUSION

The present findings demonstrated the hepatoprotective effect of *Terminalia arjuna* bark extracts on hyperlipidemic rat models. This plant can be used as hepato protecting due to the presence of various bioactive compounds such as phenolics, flavonoids, tannins etc. To explore the precise mechanism of action of specific biological active principles, further processing of the ethanolic fraction of *Terminalia arjuna* is required.

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CONFLICT OF INTERESTS

Declare None

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EFFECT OF ETHANOLIC EXTRACT OF *EMBLICA OFFICINALIS* (*AMLA*) ON PATHOPHYSIOLOGY OF LIVER IN HYPERLIPIDEMIC ALBINO WISTER RATS.

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ABSTRACT

It has been reported that hyperlipidemia plays central role in the development of atherosclerosis, liver disorders and oxidative stress. *Embilica officinalis* also known as Amla or Indian Gooseberry acts as antihyperlipidemic, antioxidant and liver tonic. It actively contains tannins, gallic acids and flavonoids. Aims: To evaluate the effect of the Ethonolic extract of *Emblica Officinalis* on pathophysiology of liver and on biochemical parameters in Hyperlipidimic albino Wister rats. Extraction of *Emblica Officinalis* by Soxhlet apparatus using 99% ethanol at 60º temp for 24hrs and Phytochemical analysis was done. Group I served as normal control. Group II Fed with Isocaloric diet. Group III Fed with Hyperlipidimic diet. Group IV. (Isocaloric diet 21 days + Embilica Officinalis 21 days). ok(hyperlipidemic diet 21 days+ *Embilica Officinalis* 21 days). Dose of ethonolic extract of *Emblica Officinalis*: (100mg/kg b. wt daily). %body weight gain, liver weight and hepatosomatic index were significantly improved in hyperlipidemic rats treated with Amla. There was significant improvement in lipid profile and markers of liver functions. Liver shown fatty changes in hyperlipidemic rats and normal Hepatocytes in Hyperlipidimic rats treated with Amla. It can be concluded that amla may be effective , natural therapeutics in hyperlipidemia and liver disorders.

KEYWORDS: ***Emblica Officinalis*, Hyperlipidimic diet, Histopathology of Fatty Liver, LFT, Lipidprofile**

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INTRODUCTION

According to the model texts Charak Samhita and Shushruta Samhita *Embilica Officinalis* regarded as "One of the best rejuvenating herbs".[1] The fruits of *Embilica Officinalis* are used as dietary and medicinal purposes by Indian system of medicine.[2] It is commonly known as Amla or Indian gooseberry. The major principles of *Embilica Officinalis* are hydrolyzable tannins (embilica A & B), Gallic acid flavonides, flavones and ascorbic acid. The *Embilica Officinalis* exerts various biological functions such as antioxidant, anti-atherosclerosis, antidiabetic, hypolipidemic, gastroprotective and cytoprotective. Along with these functions *Embilica Officinalis* also produce beneficial effects on liver functions As well as alleviate hyperlipidemia and metabolic syndrome.[3] As liver is the major organ of metabolic and energy homeostasis. Its balanced actions are over levels of endogenous metabolites such as TG, TC, HDL and glucose.[4] The hepatoprotective actions of *Embilica Officinalis* noticed to be mediated by its free radical scavenging, antioxidant and modulation of lipid metabolism.[2] In the present study we tried to or is it to prove? the effect of *Embilica Officinalis* on pathophysiology of liver of hyperlipidemic rats. We induced hyperlipidemic diet to the albino wister rats to develop an animal model of metabolic syndrome expressing fatty liver and other cardiovascular risk factors. Hyperlipidemic diets generate atherosclerosis, changes in lipids and hepatic steatosis.[5] Atherosclerosis is a disease that involves the interplay of several factors like oxidation of lipoproteins, formation of atherosclerotic plaques.[6] Amla like statins acta to inhibit HMGcoA reductase activity and ellagic acid acts to inhibit cholesterol biosyn thesis to cholesterol biosynthesis.[7] Hence the present study investigated the therapeutic efficacy of *Embilica Officinalis* extract on pathophysiology of liver of hyperlipidemic albino wister rats.

MATERIALS AND METHODS

Materials

Fresh, mature, healthy and good quality fruits of *Emblica Officinalis* (Amla) were procured from the local market, during the months of November–December 2012 identified and authenticated. Ethonolic extract preparation: 300gms of the powder of dry fruits of *Emblica Officinalis* was extracted with 99% ethanol using Soxhlet apparatus at a temperature below 60° C for 24 hours. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder.

Experimental Animals

Albino wister rats weighing 180 to 250gms were obtained from animal house of Shri B M Patil Medical college Hospital & Research Centre, Bijapur. All the five group animals were acclimatized for 7 days to the laboratory conditions at 22-24^{0}C and maintained 12 hr. light/dark cycle All the experimental procedures were performed in accordance with the approval of the Institutional Animal Ethics Committee (IAEC) of Shri B M Patil Medical college Hospital & Research Centre, Bijapur. All care was taken for animals during experimental as well as at the Time of sacrification as per the guidelines of ICMR on animal research Reference?

Experimental protocol

All the rats were divided into following five groups with 6 rats in each group. Group-I, Fed with water and ad libitum serve as control, Group II Fed with Isocaloric diet for 42 days Group-III Fed with high fat diet 42 days, Group IV fed with Ethanolic extract of Emblica Officinalis (EEO) (21 days Isocaloric diet + 21 days with EEO) and Group V Fed with high fat diet and EEO Ethonolic extract of *Emblica Officinalis* (21 days high fat diet + 21 days with EEO). It was given daily 100mg/kg B.Wt, I.P.

Sample collection and Tissue collection

All the five group animals were sacrificed by cervical dislocation at the end of the last dose after an overnight fast. After heart puncture blood was quickly collected in 10% EDTA tubes for the separation of serum. Tissue collection for histopathology: liver was isolated immediately and fixed in 10% neutral buffered formalin solution for 24 hours. The fixed tissues

were processed routinely, and then embedded in paraffin, sectioned to 3–5 µm thickness, de-paraffinized, and rehydrated using standard techniques. The extent of Hyperlipidimic diet-induced necrosis and steatosis was evaluated by assessing morphological changes in liver sections stained with hematoxylin and eosin (H&E), using standard techniques.

Gravimetry

Estimation Body Weight and Hepato-somatic Index of Albino Wister rats

The body weight of all rats was recorded at the beginning of experiment (day 1), treatment with *Emblica Officinalis* (21st day) and on the day of sacrifice (42nd day). Liver weight was measured to the nearest of 0.1 mg in a single pan balance (Digital weighing machine). Further hepatosomatic index was calculated by the formula liver weight/ total body weight.

Determination of Hematological Parameters

Hb%, RBC, WBC, Platelet, PCV & MCHC these Hematological parameters like Hb% and MCHC were evaluated in were evaluated in Sysmax -21, automated analyzer.

Biochemical Analysis

Estimation of Lipid profile

Serum triglycerides (TG), Serum total cholesterol (TC), High-density lipoprotein (HDL), Low-density lipoprotein (LDL) and Very Low-density lipoprotein (VLDL) were analyzed by MESPA automated analyzer (Method GOD POD)

Estimation of Liver Function Tests

Serum Glutamic Oxalocetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Serum Protein, Serum bilirubin, Serum albumin, Serum A/G Ratio and Serum Alkaline Phosphtase (ALP) were analyzed by Meril diagnostic Kit Method.

STATISTICAL ANALYSIS

Values are expressed as Mean ± SD. To determine the significance of inter group differences, One Way ANOVA followed by 'Post Hoc t tests' were done. P≤0.05 was considered statistically significant**.**

RESULTS

Table1

Effect of Ethanolic extract of Embilica Officinalis (Amla) on % body weight gain (on 21st day and 42nd day) on different groups of rats.

% body weight gain	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F value	P value
At 21st day	21±1.7	14.7±4.8^{a}	17.5±4.5	21.6±1.2^{b}	15.6±2.2^{d}	5.331	0.003
At 42nd day	15.6±0.7	14.7±2.4	17.1±2.4	8.6±2.6^{a,b,c}	8.8±3.4^{a,b,c}	17.6	0.00

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparision with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(* ≤0.05). GroupI is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla

Table1

At 21st day

We observed significant decrease in % body weight gain in group II compared to group I. Group IV showed significant elevation in % body weight gain compared to group II. There was significant decrease in % body weight gain in group V compared to group IV.

At 42nd day

Group IV and V depict significant decrease in % body weight gain compared to group I, II and III.

Table 2
Effect of Ethanolic extract of Embilica Officinalis (Amla) on Liver weight and Hepatosomatic index of different groups of rats.

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F Value	P value
Weight of Liver	7.73±0.17	8.87±0.95	6.72±0.36	7.67±0.22	6.68±0.83	2.246	0.093
Organosomatic Index	0.03±0.00	0.03±0.00	0.02±0.00	0.03±0.00	0.02±0.00	1.416	0.258

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparision with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(* ≤0.05). GroupI is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla

Table 2
depicts no statistical significant differences of liver weight and Hepato somatic index among all five groups of rats.

Table 3
Effect of Ethanolic extract of Emblica Officinalis on Heamatological parameters in different groups of albino wistar rats.

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F value	P value
Hb%	12.8±1.1	12.9±1.2	12.8±0.5	13.3±0.14	12±0.8	1.682	0.18
WBC	7783±2806	7266±2084	5633±1723	7383±2918	6566±1538	0.82	0.52
RBC	7.4±0.9	7.3±0.8	7.3±0.3	8±0.2	6.8±0.3[d]	2.667	0.03
Platelet	8.1±2.6	6.4±2.2	7.6±1.2	9.7±0.3	8.2±2.7	2.041	0.11
PCV	44.6±5.4	43.6±4.8	42.1±2.3	47.9±1.9	39.8±2.5[d]	3.895	0.01
MCHC	28.9±1.3	29.6±0.6	30.5±0.8	28.1±1.1[c]	30.3±1.7[d]	4.286	0.009

Values are expressed as mean±SD ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a,b,c,d express significant difference between groups. . a depicts comparision with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(* ≤0.05). GroupI is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla

Table 3
Values of Hb%, WBC and platelet have shown statistically non significant differences among all groups. RBC and PCV were significantly lower in group V compared to IV. We observed no significant relation for RBC and PCV between groups III and V. MCHC value has shown significantly higher values in group V compared to IV. Also there was significantly lower MCHC value in group IV compared to group III.

Table 4
Effect of Ethanolic Extract of Embilica Officinalis on lipid profile parameters in different groups of rats.

Parameters	GroupI	Group II	Group III	Group IV	Group V	ANOVA	
						F value	P value
TG mg/dl	160.1±20.8	140.3±35.9	200.3±93.2[a]	110±23.5[c]	129±32.2	3.60	0.01
TCmg/dl	130.6±13.6	122.8±16.1	123.1±17.5	131.5±15.8	185±17.1[a,b,c,d]	15.8	0.000
HDLmg/dl	30.3±1.86	30.0±1.89	41.8±14[a,b]	29.6±1.86[c]	36.8±2.5	4.08	0.01
LDLmg/dl	80.6±12.5	64.7±18.5	33±15.8[a,b]	79.8±12.8[c]	122.3±14.1[a,b,c,d]	27.94	0.000
VLDLmg/dl	22.7±4.2	28±7.1	44±18.6[a]	22±4.7[c]	25±6.7	3.319	0.026

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups. a depicts comparision with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(* ≤0.05). GroupI is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla.

Table 4

Levels of TG were significantly higher in group III compared to group I. Group IV showed significant decrease in TG levels compared to group III. No significant differences were observed for TG levels between group IV and group V. TC levels were significantly higher in group V compared to group I, II,III & IV. We observed significantly higher values of HDL in group III compared to group I and group II. HDL levels showed significantly lower values in group IV compared to group III. No significant differences were observed between group III and V although there were decreased in HDL levels in group V. LDL levels were significantly lower in group III compared to group I & II. We observed significantly higher values of LDL in group IV compared to group III. Also, there were significantly higher values of LDL in group V compared to Group I, II, III and IV. Group III showed significant higher levels of VLDL compared to group I. It was shown significant decrease in VLDL levels in group IV compared group III. No significant differences were observed for VLDL between group III and V.

Table 5

Effect of Ethanolic extract of Embilica Officinalis on liver parameters in different groups of albino wistar rats

Parameters	Groupl	Group II	Group III	Group IV	Group V	ANOVA	
						F value	P value
Serum bilirubin mg%	0.83±0.1	0.6±0.01	0.78±0.1	0.8±0.1	0.68±0.19	2.55	0.06
SGOT U/L	51.6±8.98	73.6±6.5	70.1±28.9	51.3±8.1	18.6±5.04[a,b,c,d]	13.5	0.00
SGPT U/L	48.1±4.83	59.5±19.7	64.5±12.5	47.16±5.67	22.83±4.26[a,b,c,d]	12.52	0.00
Serum protein gm/dl	5.68 ±0.26	5.71 ± 0.25	5.7 ±0.25	5.6 ± 0.24	6.01±0.38	1.58	0.21
Serum albumin gm/dl	2.9±0.16	2.9±0.1	3±0.19	2.86±0.13	3.4±0.34[a,b,c,d]	6.44	0.001
A/G ratio gm/dl	0.9±0.08	1±0.10	1±0.11	0.98±0.04	1.30 ±0.24[a,b,d]	6.42	0.001
Alkaline phos U/L	16.3±1.7	15.3±1.6	18.7±5	16.1±1.8	17.2±2.6	1.27	0.30

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups. . a depicts comparision with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(* ≤0.05). Group I is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla

Table 5

Serum bilirubin, serum protein and alkaline phosphate have shown statistically non significant differences among all groups. SGOT, SGPT and serum albumin levels were significantly lower in group V compared to group I, II, III and IV. A/G ratio was significantly higher in group V compared to I, II and IV

THE HISTOPATHOLOGY OF LIVER

Group I histopathology section of liver shown normal hepatic architecture compressed of hepatic lobules formed by central vein and cords of hepatocytes with indistinct sinusoidal dilatation but whereas Group II,IV& Vhas shown prominent sinusoidal dilatation (Fig 1,2,4 & 5 H&E A10X and B40X) Group III; histipathology of liver shown lobular architecture of the liver with enlarged hepatocytes containing microvescicular and macrovesicular fatty changes with sinusoidal congestion. (Fig 3 H&E A10X and B40X)

FIGURES: Showing Histopathology of Liver]

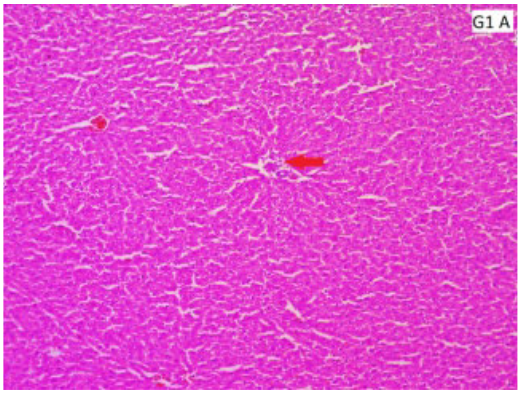

Figure 1
Group I A. Showing normal Hepatocytes in lobular pattern in 10X

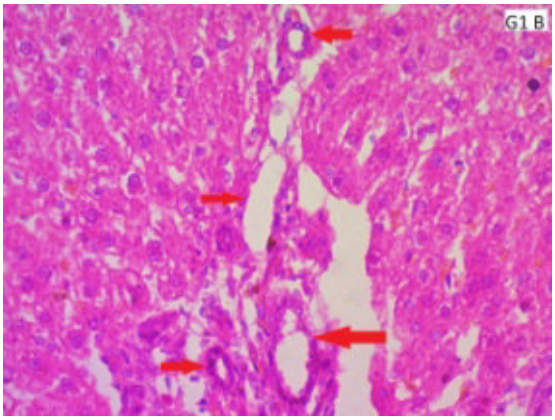

Figure 2
. Group I. B. Showing normal architecture of liver with portal tried (Bile Duct, Hepatic Artery, & Vein) in 40X

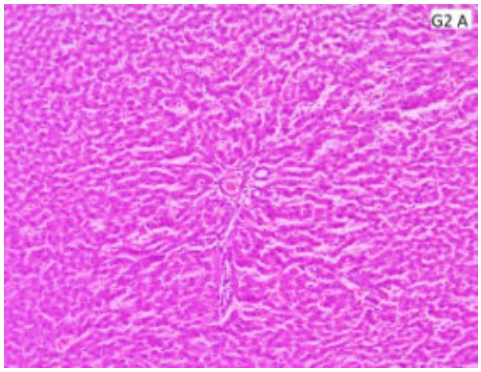

Figure 3
Group II. A. Showing normal architecture of Liver with Central vein surrounded by Hepatocytes and intervening Sinusoids in 10X.

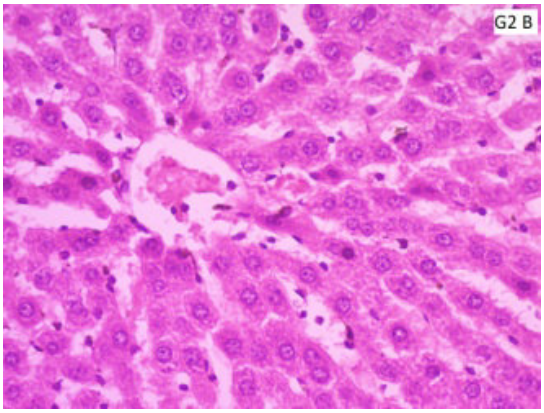

Figure 4
Group II. B. Showing normal architecture of Liver with Central vein surrounded by Hepatocytes and intervening Sinusoids in 40X

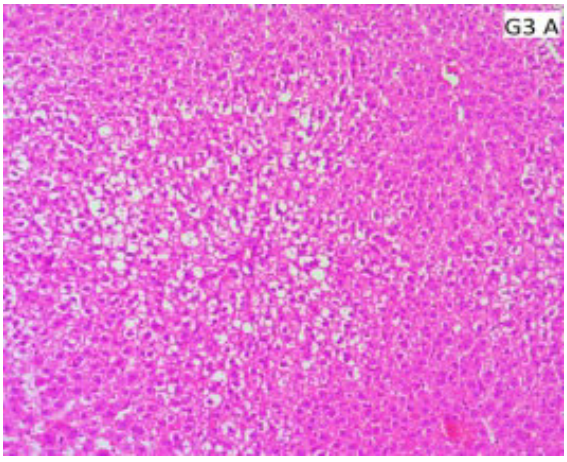

Figure 5
Group III. A. Architecture of liver showing prominent Macrovesicular & Microvesicular fatty change in 10X

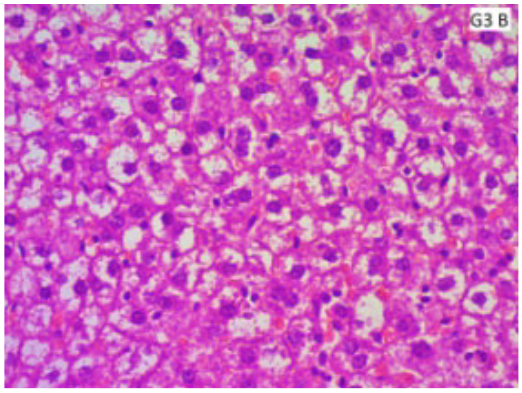

Figure 6
Group III.B. Architecture of liver showing prominent Macrovescicular & Microvescicular fatty change in 40X

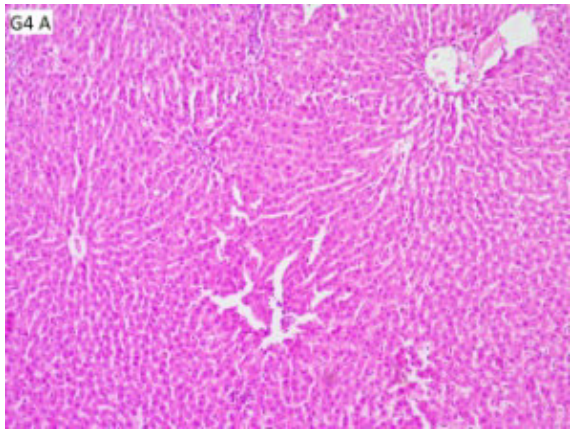

Figure 7
Group IV A. Architecture of liver showing prominent Hapatocytes separated by dilated sinusoids in 10X

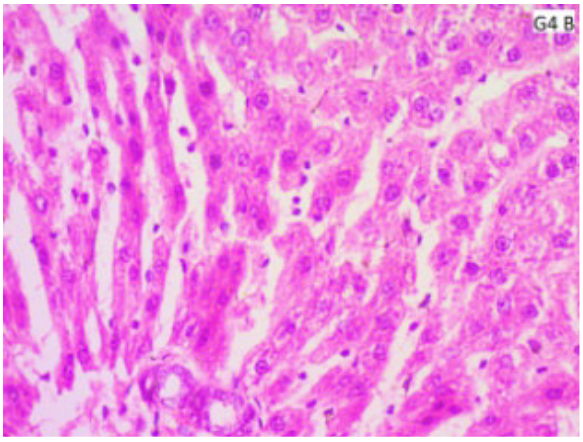

Figure 8
Group IV B. Architecture of liver showing prominent Hapatocytes separated by dilated sinusoids in 40X

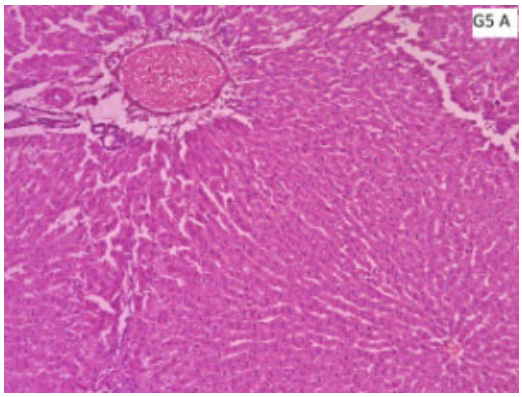

Figure 9
Group V A. Architecture of liver showing Hepatocytes separated by dilated sinusoids in 10X.

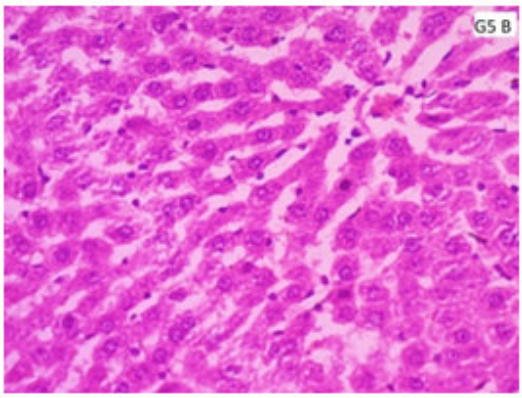

Figure 10
Group V. B. Architecture of liver showing Hepatocytes separated by dilated sinusoids in 40X

DISCUSSION

Animal models offer an appropriate mode to explore and understand the pathophysiology of disease and open the door to prevent or to treat the studied disease. Studies on such models significantly add towards enriching knowledge in the field of medical research. Hyperlipidemic animal models express clinical manifestations of fatty liver and other cardiovascular risk factors. The hyperlipidemic effects of the diet demonstrated in the increased body weight and lipid profile in albino wister rats.[5] And the hyperlipidemic rats treated with *Embilica Officinalis* showed normal body weight, lipid profile as well as normal liver histology. We observed decrease in % body weight gain in rats treated with *Embilica Officinalis* compared to Group I at 21st day. Also, there was a significant decrease in % body weight gain in rats treated with *Embilica Officinalis* compared to hyperlipidemic rats and control at 42nd day. There was no significant difference observed between hyperlipidemic rats treated with *Embilica Officinalis* and rats treated with only Embilica Officinalis at 42nd day, this could be due to excessive breakdown of tissue proteins and catabolism of fats and proteins.[8] B Antony et al, reported in their study a significant reduction in TC, LDL, VLDL & TG whereas there was significant elevation in the HDL level after treatment with *Embilica Officinalis*. In addition haemogram showed improved levels of Hb, RBC and other cells.[7] Similar results were observed in our study. Anju Lama et al showed significant increase in all the lipid parameters ($p < 0.01$) except HDL following administration of high fat diet. It was also seen that administration of the EEO at a dose of 1 gm/kg body weight along with high fat diet in the experiment animals, showed a significant decrease in all the lipid parameters ($p < 0.01$) with a significant rise in the value of HDL ($p < 0.01$).[6] We found a significant increase in levels of TC and LDL in group V (hyperlipidemic rats treated with Amla) compared to groups I, II, III and IV. SGOT & SGPT are definitive indicators of liver parenchymal injury. The enhanced levels of plasma SGOT and SGPT may be due to the leakage of these enzymes from liver cytosole to the blood stream which is the marker of hepatic toxicity.[9] Manik K Singh et al, reported no significant effect on SGOT and SGPT levels in mice treated with Amla as compared to control. In contrast, we observed significant decrease in levels of SGOT and SGPT in group V(Hyperlipidemic rats treated with Amla) compared to group I, II, III and IV.[10] Rupal A Vasant et al observed significantly reduced plasma ALP levels in the rats treated with Amla (FEo 2.5,FEo 5, FEo 10) compared to normal control and fluoride control groups. In our study, we have shown no significant plasma ALP levels in group V (Hyperlipidemic rats treated with Amla) compared to group I, II, III and IV.[11] In our study, histopathological

analysis at the end of 3 weeks showed increased fat deposition in liver of rats fed with hyperlipidemic diet Group II Similar observation was reported by Jasmine Bathera et al in their study. They showed hepatocellular fat deposition and ballooning in the liver samples of high fat fed hamsters compared to control hamsters. Control hamsters showed normal liver histology.[5] Jitendra Kumar et al, reported significant decrease in albumin concentration in Amla treated chickens compared to control. In contrast, our study has shown significant increase in serum albumin in group V(Hyperlipidemic diet + Amla treatment)compared to all four groups. They have also shown significant increase in levels of serum protein and non significant reduction of A/G ratio in Amla treated group as compared to control group.[12] Whereas our study has shown significant increase in A/G ratio in group V compared to group I, II and IV. The liver samples of Group III Hyperlipidimic rats showed the lobular architecture with enlarged Hepatocytes containing microvesicular and macrovesicular fatty changes with sinusoidal congestion in (Fig 5. Group III A. H&E 10X, Fig 6 group 3.B H&E 40X). Administration of Hyperlipidimic diet with *Emblica Officinalis* showed near normal appearance of Hepatocytes (Fig. 9. Group V A H&E 10X, Fig 10. Group V.B. H&E 40X). Similar observation was found in V. Damodara Reddy et al study.[13] Although the exact mechanism by which amla exerts this beneficial effect is presently not clear, it brings about favorable changes in the lipid profile via several mechanisms, including interference with cholesterol absorption, inhibition of HMG-CoA reductase activity, and an increase in lecithin cholesterol acyl transferase activity.[14] The mechanism by which *Embilica Officinalis* exerts its beneficial effects may be like Statins. *Embilica Officinalis* is containing phenolic groups like Tannins, Gallic acid which act like Statin. Like Statin, *Embilica Officinalis* inhibits HMG CoA reductase activity. Ellgitannins and Ellagic acid obtained on hydrolysis of tannins inhibits epoxidase enzyme, a rate limiting enzyme of cholesterol biosynthesis.[7] *Embilica Officinalis* contains many liver tonic which can be used against acute viral hepatitis and other liver disorders.[15] In our study we tried to investigate positive influence of *Emblica Officinalis* on pathophysiology of liver of hyperlipidemic albino wister rats. We observed *Embilica Officinalis* useful in regulating hyperlipidemia and pathophysiology of liver in albino wister rats fed with hyperlipidemic diet.

CONCLUSION

It can be concluded from our study that *Embilica Officinalis* may be good, natural potential therapeutics for Dyslipidemia and hepatic dysfunction. However extensive clinical studies are required in large numbers of patients to establish the efficacy and safety *of Embilia Officinalis* in the management of Dyslipidemia and related disorders like atherosclerosis and hepatic dysfunction.

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ORIGINAL ARTICLE

Effect of Ethanolic Extract of *Emblica officinalis* on Histopathology of Kidney and on Biochemical Parameters in Hyperlipidemic Albino Rats

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Abstract:

Background: It has been reported that hyperlipidemia plays a central role in the development of atherosclerosis and oxidative stress. *Embilica officinalis* also known as Amla or Indian Gooseberry acts as antihyperlipidemic and antioxidant. Its active ingredients contains tannins, gallic acid and flavonoids. *Aim & Objectives:* It was aimed to evaluate the effect of ethanolic extract of *Emblica officinalis* on histopathology of kidney and on biochemical parameters in hyperlipidemic albino Wistar rats. *Material and Methods:* Extraction of dried fruits of *Emblica officinalis* was done by Soxhlet apparatus using 99% ethanol at 60^0C for 24 hours and also phytochemical analysis was done. Group I served as normal control. Group II was fed with isocaloric diet. Group III was fed with hyperlipidemic diet. Group IV was fed with isocaloric diet for 21 days + *Embilica officinalis* for 21 days. Group V was fed with hyperlipidemic diet for 21 days+ *Embilica officinalis* for 21 days. The dose of ethanolic extract of *Emblica officinalis* was taken as 100mg/kg body weight daily. *Results:* Percent body weight gain, kidney weight and nephro-somatic index significantly improved in hyperlipidemic rats treated with *Emblica officinalis*. There was a significant improvement in serum electrolyte and kidney markers. It was found that there were focal glomerular lesions with thickening of glomerulus in the kidneys of rats on hyperlipidemic diet and normal renal histology of rats on hyperlipidemic diet treated with *Emblica officinalis*. *Conclusion:* It can be concluded that *Emblica officinalis* may be a good, natural therapeutic agent against hyperlipidemic diet induced oxidative damage and nephrotoxicity.

Keywords: *Emblica officinalis*, Hyperlipidemic diet, Histopathology of Kidney, Kidney markers, Nephrotoxicity, Serum electrolyte

Introduction:

Medicinal plants are nature's gift for human beings to boost a disease free healthy life. Various medicinal plants are present in a class of herbal preparations of the Indian medicine system [1]. Among many of these herbal drugs *Embilica officinalis* is one of the precious herbal drug, commonly known as Indian gooseberry or Amla which belongs to the family of Euphorbiacae. Amla is very rich in nutrition and can be an important dietary source of Vitamin C, minerals and amino acids etc [2]. The plant also contains phenolic compounds such as tannins, saponins, phyllembelin and embilicanin. The fruit of Amla shows antioxidant, antidiabetic, hypolipidemic, antibacterial and hepatoprotective properties [3]. Along with these functions *Embilica officinalis* also may produce beneficial effects on kidney functions. Kidney contributes major role in electrolyte balance and Blood Pressure (BP) regulation. BP is regulated by renal handling of substances like Na^+, Cl^- and $HCO3^-$. It happens

under the control of renin angiotensin mechanism. In this way it maintains homeostasis of the body [4]. In the present study we tried to find out the effect of *Embilica officinalis* on histopathology of kidney in hyperlipidemic rats. We have given a hyperlipidemic diet to induce hyperlipidemia in albino Wistar rats to develop an animal model expressing changes in kidneys and kidney markers (serum creatinine and blood urea). High fat diets lead to atherosclerosis. It is a condition that involves the interplay of several factors like oxidation of lipoproteins and atherosclerotic plaques formation [5]. This pathogenesis could induce renal vasoconstriction followed by hypoxic condition in the kidney which promotes further ischemic renal injury. The hypoxic tissue produces reactive oxygen species (ROS) more than antioxidant present in the renal tissue. Amla as an antioxidant contains gallic acid which is a multiple hydroxyl group compound. It donates its proton to break the chain reaction of free radicals acting as an inhibitor to lipid peroxidation [6]. It was aimed to explore the nephroprotective effect of ethanolic extract of *Embilica officinalis* on renal dysfunction and pathological destructions in a hyperlipidemic rat model.

Material and Methods

Materials:

Healthy and good quality fresh, mature, *Emblica officinalis* (Amla) fruits were procured from the market, in the months of November–December 2012, and were identified and authenticated by the Department of Botany K.C.P. Science College, Bijapur. Extraction process was conducted in the Department of Pharmacology, BLDEA college of Pharmacy, Bijapur.

Extraction:

300gms of the powder of dried fruits of *Emblica officinalis* (Amla) was extracted with 99% ethanol using Soxhlet apparatus at a temperature 60° C for 24 hours. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder.

Study design:

Experimental Animals:

Albino Wistar rats weighing 180 to 250gms were obtained from animal house of Shri B M Patil Medical college Hospital and Research Centre, Bijapur. All animals were acclimatized for 7 days to the laboratory conditions at 22-24^{0}C maintaining a 12- hour light/dark cycle. All the experimental procedures were performed in accordance with the approval of the Institutional Animal Ethics Committee (IAEC) of Shri B M Patil Medical college Hospital and Research Centre, Bijapur. All care of animals was taken as per the guidelines of ICMR on animal research (2006) during the experiment as well as at the time of sacrifice. This study was undertaken in the Department of Anatomy. The newborn and old and diseased rats were excluded.

Preparation of Isocaloric Diet:

For 1kg of diet, 180gm of casein, 620gm of carbohydrate, 200gm of fat and 1% of multi vitamin and 2% NaCl were taken [7].

Preparation of Hyperlipidemic Diet:

For 1kg of diet, 180gm of casein, 520gm of carbohydrate, 300gm of fat and 1% of multi vitamin and 2% NaCl were taken [8].

Experimental Protocol

All the rats were divided into following five groups with 6 rats in each group.

Group I served as normal control fed with water and food *ad libitum*, Group II was fed with isocaloric diet for 42 days, Group-III was fed with high fat diet for 42 days, Group IV was fed with isocaloric diet for 21 days and ethanolic extract of *Emblica officinalis* (EEO) for 21 days each and Group V was fed with hyperlipidimic diet for 21 days and EEO for 21 days each. 100mg/kg body weight of EEO was given daily. [9]

Sample collection

Every alternate week (six rats) one group of animals were sacrificed by cervical dislocation at the end of the last dose with an overnight fast. Blood was collected in normal tubes for the separation of serum, by doing retro-orbital puncture, before sacrificing the animals.

Tissue collection for histopathology:

After proper dissection of animal kidneys were isolated immediately and fixed in 10% neutral buffered formalin solution for 24 hours [10]. The fixed tissues were processed routinely and then embedded in paraffin, sectioned to 3–5 µm thickness, de-paraffinized, and rehydrated using standard techniques. The extent of hyperlipidemic (high fat diet) induced necrosis was evaluated by assessing morphological changes in kidney sections stained with Hematoxylin and eosin (HandE), using standard techniques.

Gravimetry:

Estimation of Body Weight and Renal-somatic Index of Albino Wister rats:

Procedure 1:

The total body weight of each rat was recorded on the first day (beginning of experiment), Group I was fed with water and food *ad libitum*, Group III and V were fed with hyperlipidemic diet and Group II and IV were fed with iso-caloric diet for 21days and the weight was measured. The same diet was continued from 21st to 42nd day. In addition Group IV and V were treated with EEO.

Procedure 2:

After 42nd day again weight was measured and after overnight fast rats were sacrificed. With proper dissection right side kidney was collected and weight was measured to the nearest of 0.1 mg on a digital weighing machine. Further the renal-somatic index was calculated by the formula of kidney weight/ total body weight.

Estimation of Biochemical Parameters (Renal Markers and Serum Electrolytes):

Blood Urea, Serum Creatinine, Serum Na^+ (Sodium), K^+(Potassium), Ca^{++} (Calcium) and Cl^- were analyzed by Meril Diagnostic Kit Method.

Statistical Analysis:

Values were expressed as Mean ± SD. To determine the significance of inter group differences, one way ANOVA followed by 'Post Hoc t tests' were used. $P \leqslant 0.05$ was considered statistically significant**.**

Results:

Gravimetry

At 21st day: It was observed that there was a significant increase in percent body weight gain in Group III as compared to Group I ($p< 0.05$). Group IV showed a significant decrease in % body weight gain as compared to Group III ($p< 0.05$).

Table 1: Effect of Ethanolic Extract of *Emblica officinalis* on Percent Body Weight Gain

Groups	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F Value	P value
On 21st day	14.2 ± 4.7	18.5 ± 4.1	21±1.0^{a}	15±3.8^{c}	19.8±1.7	5.2	0.0061
On 42nd day	14.5 ± 2	16.8 ± 1.4	8.9±3^{a,b}	8.9±3^{a,b}	14.6±2.1^{c,d}	12.2	0

*Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups, a depicts comparison with Group I, b depicts comparison with Group II, c depicts comparison with Group III and d depicts comparison with Group V (*P value is⩽ 0.05). Group I -normal control rats, Group II - Isocaloric diet fed rats, Group III- hyperlipidemic diet fed rats, Group IV- EEO fed rats, Group V-hyperlipidemic + EEO fed rats*

At 42nd day: It was observed that there was a significant decrease in percent body weight gain in Group III and IV as compared to Group I and II ($p< 0.05$). Group V showed a significant increase in percent body weight gain as compared to Group III and IV ($p<0.05$).

It was observed that there was significant decrease in body weight of Group II compared to Group I ($p<0.05$). There was significant increase in body weight of Group III compared to Group II ($p<0.05$). Kidney weights of Group II and Group III were lower compared to group I. Group IV which showed significant increase in kidney weight compared to Group II and Group III. Group V showed a significant relation with Groups I, II, III and IV ($p<0.05$). There was a significant decrease in renal somatic index of Group II and Group III compared to Group I. Group V showed significant relation of renal somatic index to Group II and III ($p<0.05$).

Table 2: Effect of Ethanolic Extract of *Emblica officinalis* on Renal Somatic Index

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F Value	P value
Body weight (gm)	253 ± 11.4	228 ± 19.3[a]	254 ± 9.6[b]	236 ± 6.2	234 ± 10.2	6.24	0.002
Kidney weight (gm)	2.6 ± 0.1	0.8 ± 0.1[a]	0.6 ± 0.09[a]	2.5 ± 0.1[b,c]	2.1 ± 0.2[a,b,c,d]	223.1	0
Renal somatic Index	0.01 ± 0.0	0.003 ± 0.0[a]	0.002 ± 0.00[a]	0.01 ± 0.0[b,c]	0.009 ± 0.0[b,c]	229.3	0

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups, a depicts comparison with Group I, b depicts comparison with Group II, c depicts comparison with Group III and d depicts comparison with Group V (P value is⩽ 0.05). Group I -normal control rats, Group II - Isocaloric diet fed rats, Group III- hyperlipidemic diet fed rats, Group IV- EEO fed rats, Group V-hyperlipidemic + EEO fed rats*

Table 3: Effect of Ethanolic Extract of *Emblica officinalis* on Kidney Markers and Serum Electrolytes

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F Value	P value
Blood Urea (mg%)	31±4.1	28±6.0	30±6.2	31±5.2	27±7.0	0.7	0.6717
S. Creatinine (mg%)	0.9±0.1	0.8±0.2	0.7±0.3	0.9±0.1	0.8±0.2	0.7	0.376
Na^+ (mEq/L)	139±2.0	143±5.0	140±1.5	139±1.8	141±4.9	2.89	0.2514
K^+ (mEq/L)	4.9±0.7	6.4±0.6[a]	4.6±1[b]	4.8±0.7[b]	4.7±0.6b	4.03	0
Ca^{++} (mg/dl)	8.6±0.08	9.9±0.5[a]	8.7±0.1[b]	8.6±0.1[b]	8.7±0.2b	13.2	0
Cl^- (mEq/L)	98±0.9	97±0.9	97±1.3	97±1.2	98±1.2	0.5	0.2459

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups, a depicts comparison with Group I, b depicts comparison with Group II, c depicts comparison with Group III and d depicts comparison with Group V (P value is⩽ 0.05). Group I -normal control rats, Group II - Isocaloric diet fed rats, Group III- hyperlipidemic diet fed rats, Group IV- EEO fed rats, Group V-hyperlipidemic + EEO fed rats*

Biochemical Parameters:

No significant differences for Blood Urea, Serum Creatinine and Serum Cl^- among all Groups was observed. It is observed that the values for Na^+, K^+, Ca^{++} were all significantly higher for Group II. All the other groups had similar values.

Histopathology of Kidney:

Photomicrography of H and E stained kidney of Group III hyperlipidemic (high fat diet) rats showed focal glomerular lesions including thickening of the glomerulus and normal renal tubules, whereas Group I normal control, Group II isocaloric diet, Group IV isocaloric diet with EEO and Group V hyperlipidemic diet with EEO showed normal microscopic architecture of kidney.

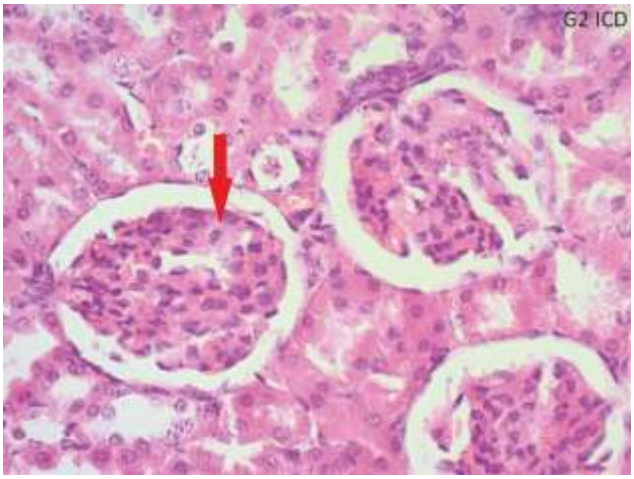

Fig. 2: Iso-caloric Group II Rat Kidney showing Normal Architecture of Kidney and No Histopathologic changes (H and E 40X)

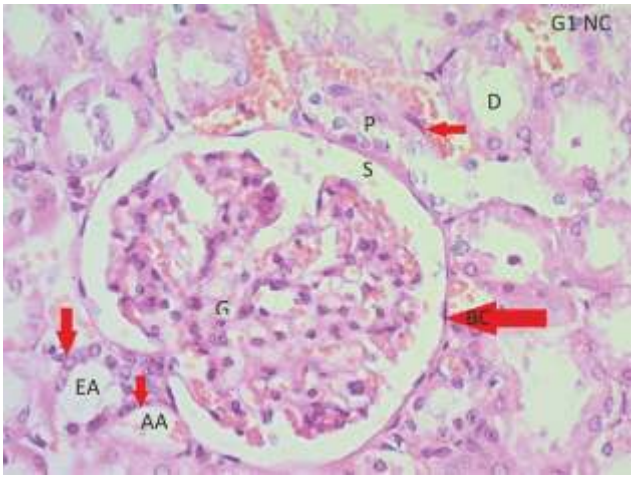

Fig. 1: Control Group showing Normal Parenchyma and No Histopathologic Changes (H and E satin 40X) G-Glomerular, S-Space of Urine, BC- Bowmen's Capsule, P-Proximal tubule, D-Distal tubule, EA- Efferent arteriole, AA- Afferent Arteriole

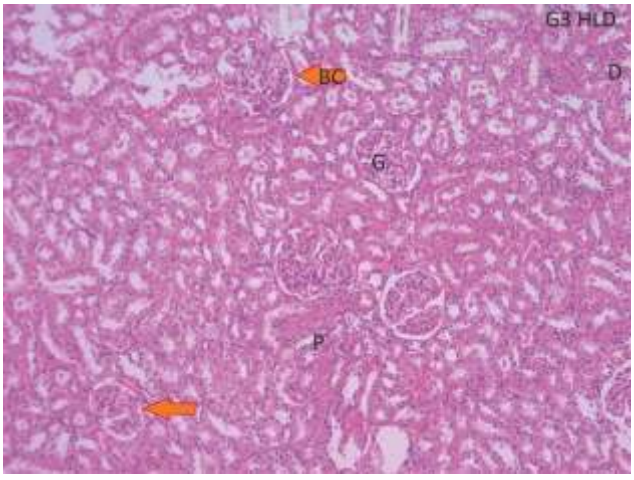

Fig. 3: H and E stained 10 X kidney of Group III Hyperlipidemic (High fat diet) Rats showing Focal Glomerular Lesions including Thickening of the Glomerular Microscopy. G-Glomerular, BC- Bowmen's Capsule, P-Proximal Tubule, D-Distal Tubule

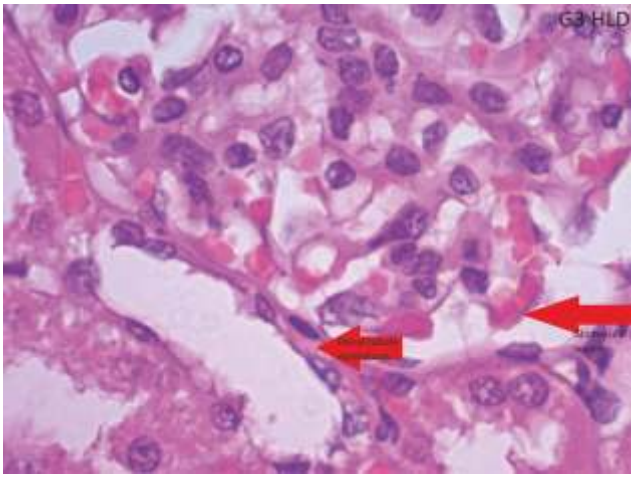

Fig. 4: Histopathology of H and E Stained 100 X Kidneys of Group III Hyperlipidemic (High Fat Diet) Rats showing Focal Glomerular Lesions Including Thickening of the Glomerulus and Normal Renal (DCT) Tubules

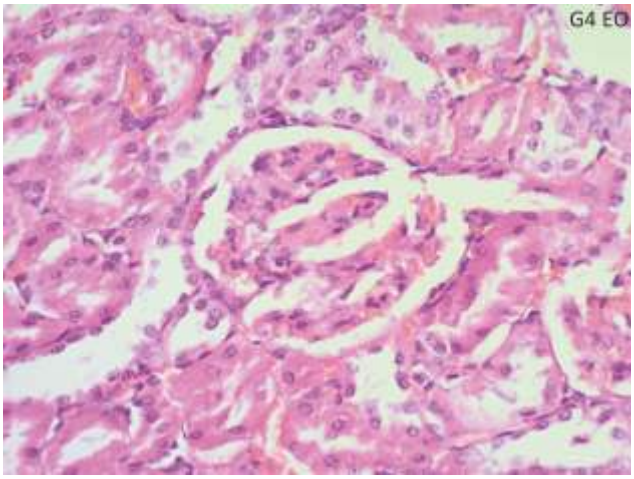

Fig.6: Rat Kidney of Group IV Showing Normal Architecture of Kidney and No Histopathological Changes (HandE 40X)

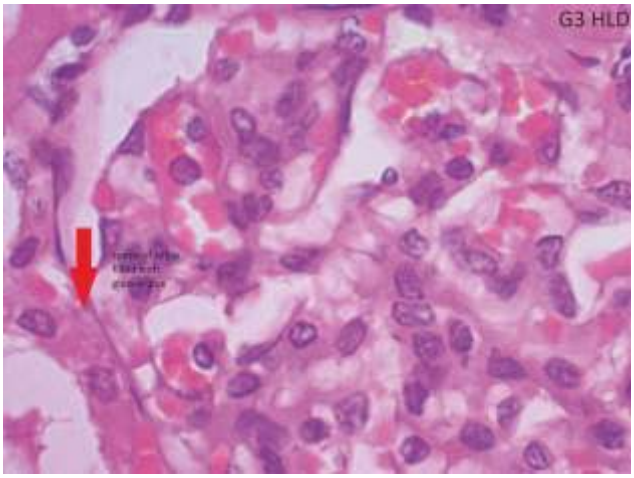

Fig. 5: Histopathology of H and E Stained 100X Kidney of Group III Hyperlipidemic (High Fat Diet) Rats showing Glomerular Thickening

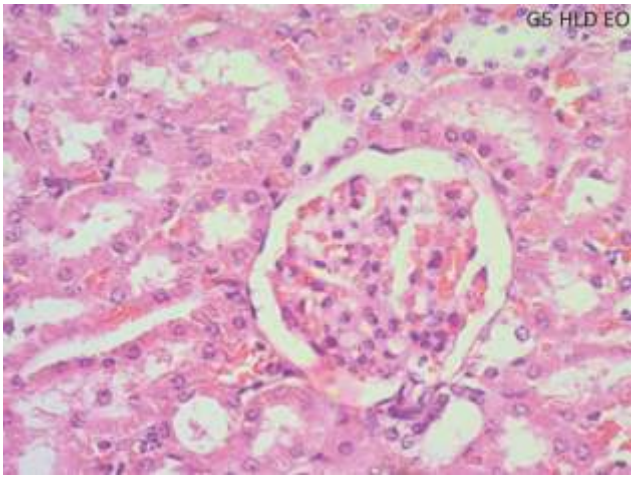

Fig.7: H and E stained (40 X) Kidney of Group V Hyperlipidemic (High fat diet) Rats Treated with EEO showed Histopathological Changes

Discussion:

The study was aimed to demonstrate the nephroprotective effect of EEO to prevent the development of renal dysfunction and alteration of histopathology of kidney which are assessed by biochemical and renal markers in a hyper-lipidemic rat model. Studies on such models significantly add to the knowledge towards enriching in the field of medical research. Hyperlipidemic animal models expressed changes in renal markers, biochemical parameters and histopathology of kidney. As *Emblica officinalis* being a potent antioxidant it exerts free radical scavenging activity and shows preventive role against fat induced renal toxicity [11].

It was observed no histopathologic change and no alteration of renal markers were found in rat kidneys of Group V and they also retained normal body weight. It indicates that EEO has exerted nephroprotective effect in rat kidney even when it was given hyperlipidemic diet. EEO has revealed to attenuate hyperlipidemia in Group V. EEO may be helpful to restore renal functioning due to its presence of gallic acid and tannins [6]. An increase in percent body weight gain in rats treated with EEO (Group IV) was observed compared to control rats (Group I) at 21st day ($p<0.05$). Also there was a significant increase in percent body weight gain in rats treated with EEO (Group IV) compared to hyperlipidemic rats treated with EEO (Group V) at 42nd day ($p<0.05$). There was a significant decrease in percent body weight gain in rats treated with *Embilica officinalis* (Group IV) compared to control rats at 42nd day ($p<0.05$).

Adis *et al* found that serum creatinine and bilirubin levels increased significantly in contrast media group compared to control group. Pretreatment with EEO demonstrated its nephroprotective effect by attenuating the severity of renal pathological damage and improving renal functioning [6]. In contrast we found non significant decrease in serum creatinine and blood urea in Group V compared to Group I. Eteng *et al* showed a slight non significant increase in serum (Na+) level for Group I (100mg/kg body weight) Wistar rats [12].

We observed that Na^+ and K^+ levels were significantly lower in Group III, IV and V compared to Group II ($p<0.05$). Also there was significant increase in levels of K^+ in group II compared to Group I ($p<0.05$). Ca^{++} level was significantly higher in Group II compared to Group I ($p<0.05$). Increased serum levels of triglycerides and cholesterol causes to lipid accumulation in different organs and tissues like arterial wall, liver, kidney and muscles [13]. In our study we observed the effect of (high fat diet) hyperlipidemia on the kidneys of albino Wistar rats. The hyperlipidemia caused minimum percent of lipid accumulation in kidney tissue. Histo-pathology of renal corpuscles exhibited normal appearance in Groups I and II (Fig.1, Fig.2) whereas Group III showed the prominent increase in the glomerular capillaries (Fig. 3, 4 and 5). Microscopic study of high fat diet i.e. Group III rat kidney showed focal glomerular lesions including thickening of glomerular changes and completely decreased space (space for urine) between glomerulus and Bowmen's capsule. Epithelial cell lining of renal tubules were hemorrhagic and appeared dilated compared to normal histology of kidney. Fig. 6 and 7 both show normal histopathology, Fig. 4 and 5 show changes due to hyperlipidemia and Fig. 7 shows normal histopathology after high fat diet and treatment with EEO.

Experimental studies have shown that hyperlipidemic diet may be associated with increased oxidative stress in animals [14].

In our study we tried to assess the influences of *Emblica officinalis* on renal functions and histopathology in hyperlipidemic albino Wistar rats. From the results, *Embilica officinalis* is useful in regulating hyperlipidemia and

histopathology of kidneys in albino Wistar rats fed with hyperlipidemic diet.

Conclusion:

In conclusion, we describe hereby distinctive renal glomerular ultrastructural injury associated with hyperlipidemia and markedly reduced alteration in renal glomerular histopathology when treated with fruit extract of *Emblica officinalis*. We would like to state that fruit extract of *Embilica officinalis* plays a protective role against hyperlipidemic diet induced oxidative damage and nephrotoxicity. The mechanistic pathways by which *Embilica officinalis* acts as a protective therapeutic against that particular toxin is not fully clear except its antioxidant property. Further investigations are necessary to find the active functional group(s) of the compound(s) of *Embilica officinalis*. However extensive clinical studies are required to establish the efficacy and safety of *Embilica officinalis* for treating multiple pathologies associated with hyperlipidemia.

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Paper presentations in Conference and Seminars

ORAL PRESENTATION

1. **14th Karnataka state Anatomy Conference at Shri B M Patil College, Hospital & Research Centre, BLDE University Bijapur 8-9th September 2012.**

   Topic: Effect of Terminalia Arjuna and Emblica Officinalis Extract on Cardiovascular System in Albino Wister Rats

POSTER PRESENTATIONS

1. **Application to Nanotechnology in Health Care June 13, 2012 at BLDEA's College of Pharmacy, Bijapur**
   Topic: Effect of Terminalia Arjuna and Emblica Officinalis Extract on Cardiovascular System in Albino Wister Rats

2. **International Congress on Ayurvedic Concepts and treatment of Malignant Disorders: at SDM College of Ayurvedic Sciences UJRE Udupi. Karnataka.**
   15-16th December 2012.
   Topic: Effect of Terminalia Arjuna and Emblica Officinalis Extract on Cardiovascular System in Albino Wister Rats

3. **14th Karnataka state Anatomy Conference at Shri B M Patil College, Hospital & Research Centre, BLDE University Bijapur 8-9th September 2012.**
   Topic: Effect of Terminalia Arjuna and Emblica Officinalis Extract on Cardiovascular System in Albino Wister Rats

From

Dr.R.S. Wali
Chairman,
Institutional Animal Ethics Committee (IAEC),
Prof. & HOD, Dept. of Pharmacology,
BLDEU's Shri. B.M.Patil Medical College,
BIJAPUR.

To,

Dr. Bheemshetty S Patil,
Lecturer, Dept. of Anatomy,
BLDEU's Shri. B.M.Patil Medical College,
BIJAPUR.

ETHICAL CLEARANCE CERTIFICATE

The Institutional Animal Ethics Committee (IAEC) of this College met on 31.05.2011 at 10.30am to scrutinize the Research Project submitted by faculty member of this College.

After scrutiny the following research project has been accorded ethical clearance

Title: "Effect of Terminalia Arjuna and Emblica Officinalis extract on cardiovascular system in albino wister rats"

Principal Investigator: Dr. Bheemshetty S Patil, Lecturer, Dept. of Anatomy.

Dr. R. S. Wali
Chairman, (IAEC)
Prof. & HOD,
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BLDEU's Shri. B. M. Patil
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Medical College BIJAPUR.

EFFECT OF TERMINALIA ARJUNA AND EMBLICA OFFICINALIS EXTRACT ON CARDIOVASCULAR SYSTEM IN ALBINO WISTER RATS